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Short communication

Detection of an animal-derived G4P[6] group A rotavirus strain in a symptomatic child, in Italy

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ABSTRACT

During 2017, a G4P[6] group A rotavirus strain was identified in the feces of an Italian child hospitalized for acute gastroenteritis in Southern Italy. Nucleotide sequencing of the 11 genomic segments, revealed the G4-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1 genotype constellation. Phylogenetic analyses of the gene segments investigated revealed high nucleotide sequence identities with G4P[6] RVA strains detected previously in pigs and in humans. The human strains related to the Italian G4P[6] were mainly reported from Asia, and were detected after an inter-species transmission event from swine.

This study reports the genotyping and phylogenetic analysis of a G4P[6] RVA strain presenting a genomic constellation never detected before in Italy. In addition, this strain was able to cause AGE symptoms in a healthy child, successively hospitalized.

The molecular characterization suggested zoonotic origin and inter-species transmission of this strain from swine, living open the possibility of its importation from abroad.

Group A rotaviruses (RVAs) are the leading cause of acute gastroenteritis (AGE) in children < 5 years of age worldwide, causing ≈250,000 deaths annually (Tate et al., 2016). The RVA genome is composed of 11 double-stranded RNA segments, encoding 6 structural viral (VP) and 5 nonstructural (NSP) proteins (Desselberger, 2014). The nucleotide sequence differences of genes encoding the VP7 and VP4 proteins define the G- and P- genotypes, with a binary classification system. To date, 36 G and 51 P genotypes have been identified, but most RVA human infections worldwide are related to 6 major genotypes: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] (Doro et al., 2014). Genome segment reassortment between human strains or human and animal strains during co-infections can generate viruses with novel genotype combinations, possibly generating emerging RVA variants (Nakagomi and Nakagomi, 2002; Martella et al., 2010; McDonald et al., 2016).

Since 2008 a more complete genotyping system has been introduced, defining genotypes for all the 11 genomic segments. Several studies have defined preferred genomic constellation in humans, with two major (Wa-like RVA strains, presenting a GX-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 genotype constellation 1; and DS-1 like RVA strains, GX-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2 constellation 2) and one minor genomic constellations (AU1-like strains, G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3, constellation 3) (Matthijnssens and Van Ranst, 2012). This genotyping system allowed to identify possible reassortment events, defining better the origin of the RVA strains.

Since 2007, the RVA surveillance network RotaNet-Italy has performed the monitoring of the circulating RVA genotypes, showing a massive circulation of common genotypes among children, together with sporadic detections of uncommon, exotic, or zoonotic genotypes. This paper reports the detection of a G4P[6] RVA strain causing AGE symptoms in an Italian child in Southern Italy. The critical dehydration status of the patient required hospitalization.

In August 2017, in the framework of the RotaNet-Italy surveillance, the stool specimen of an Italian 6 months old male child hospitalized in Potenza (Basilicata, Southern Italy) for AGE (only diarrhea with > 3 discharges per day) was collected and sent to the Istituto Superiore di Sanità of Rome for genotyping. Clinical information were obtained from the admitting pediatric unit in compliance with the Italian Guidelines on Informed Consensus.

Total viral RNA was extracted from 140 µl of 10% fecal suspensions in H_2O, using the Viral RNeasy Mini Kit (Qiagen/Westburg, Milano, Italy), according to the manufacturers' instructions.

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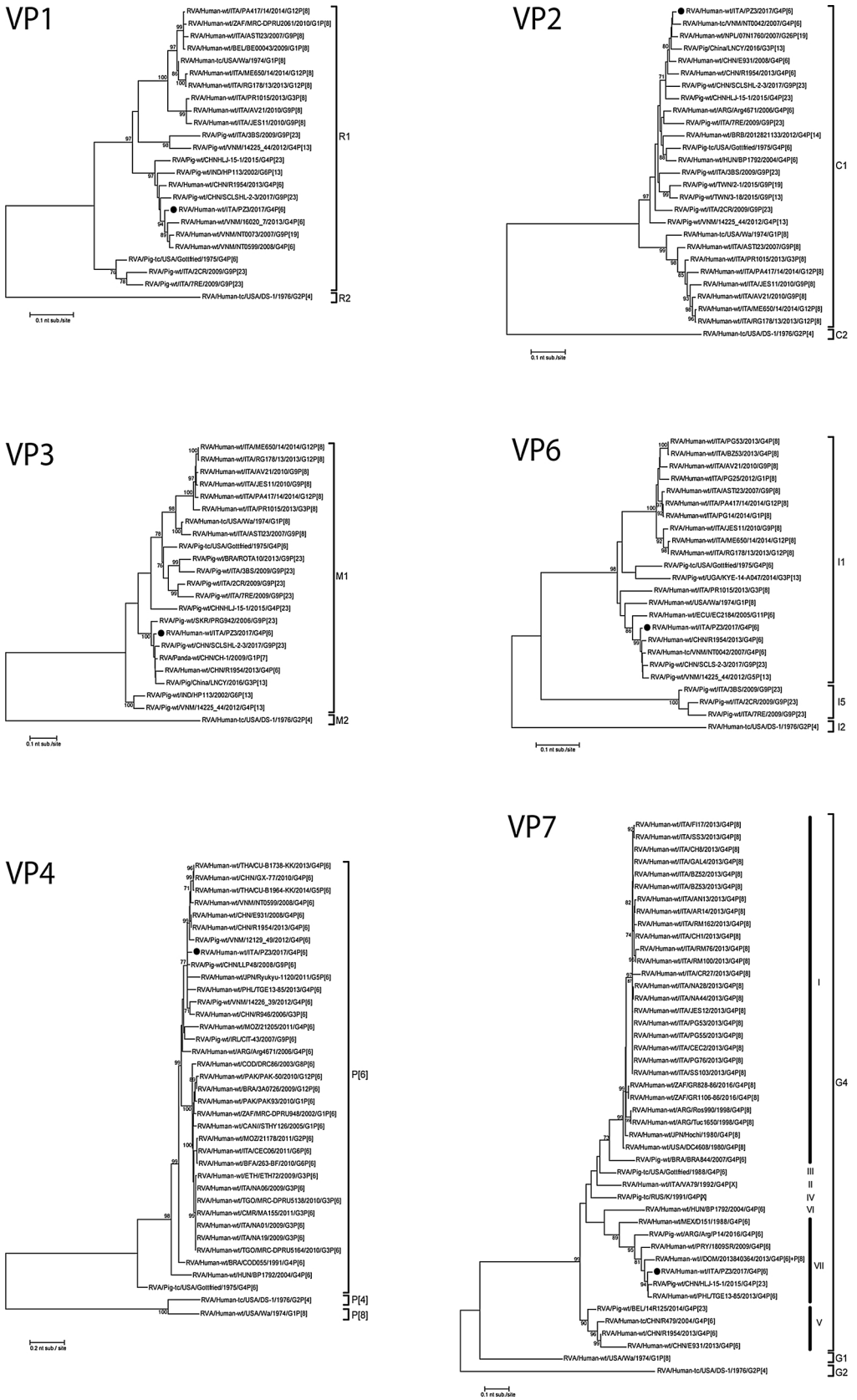

Fig. 1. Phylogenetic trees based on the partial ORF of genes coding for structural proteins: VP1 (nt 44-911), VP2 (nt 52-833), VP3 (nt 51-888), VP4 (nt 201-791), VP6 (nt 119-948), and VP7 (nt 163-760). Italian G4P[6] PZ3/2017 strain is highlighted with a filled circle. Trees were built with the maximum likelihood method (ML), and bootstrapped with 1000 repetitions; bootstrap values below 70 are not shown.

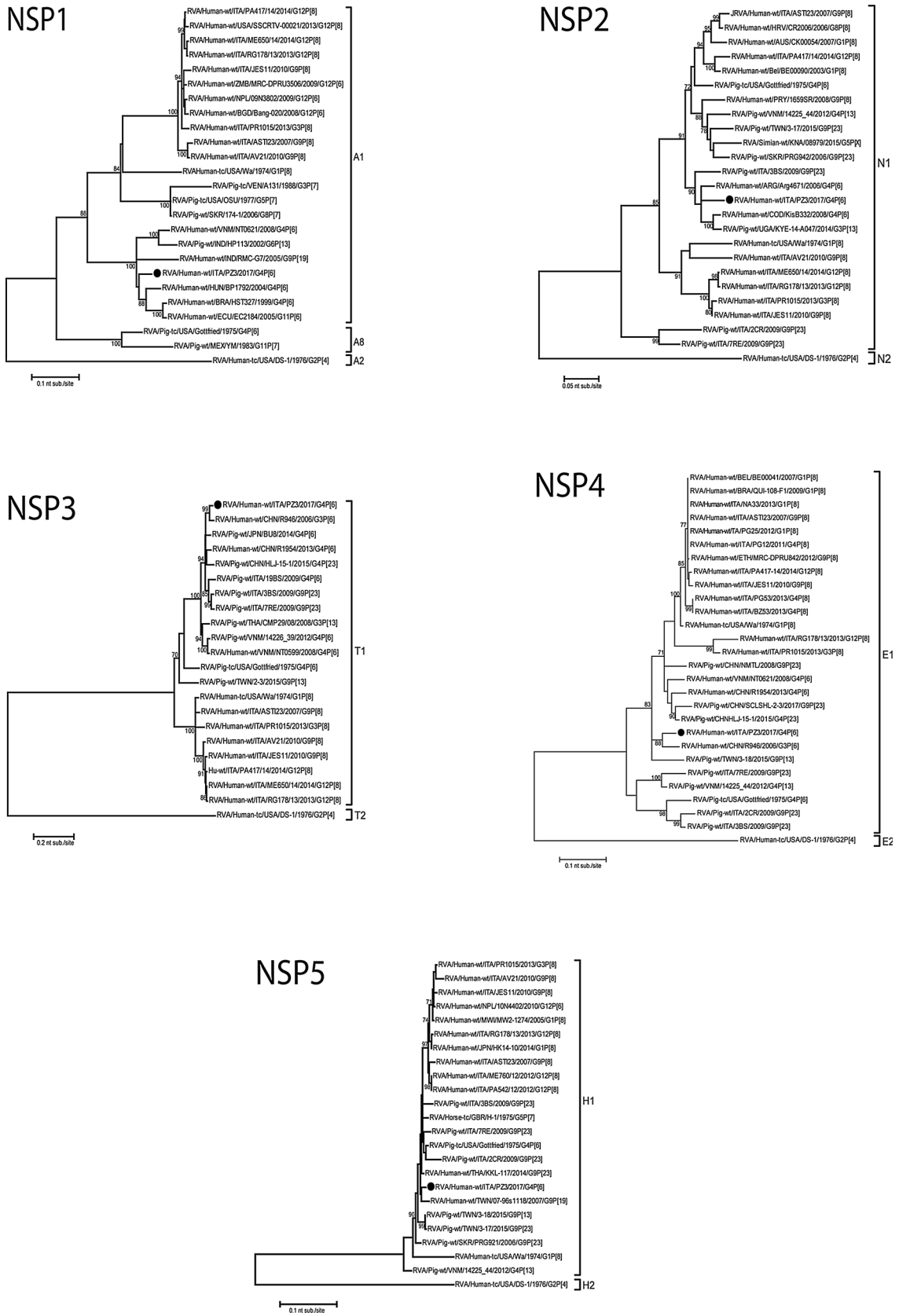

Fig. 2. Phylogenetic trees based on the partial ORF of genes coding for non-structural proteins: NSP1 (nt 51-856), NSP2 (nt 51-1000), NSP3 (nt 129-967), NSP4 (nt 149-569), and NSP5 (nt 45-615). Italian G4P[6] PZ3/2017 strain is highlighted with a filled circle. Trees were built with the maximum likelihood method (ML), and bootstrapped with 1000 repetitions; bootstrap values below 70 are not shown.

G- and P-genotyping were performed by reverse transcription nested polymerase chain reaction (RT-nPCR), using mixtures of primers for VP7 or VP4 different genotypes, as previously described (Gentsch et al., 1992; Iturriza-Gomara et al., 2004).

After genotyping, the RVA strain RVA/Human-wt/ITA/PZ3/2017 revealed an uncommon G4P[6] genotype, never detected before in Italy. For this reason, strain PZ3/2017 was subjected to nucleotide sequencing of the 11 genomic segments in order to define the complete viral genomic constellation.

For nucleotide sequencing, RT-PCR reactions included primers specific for each of the 11 genes (Esona et al., 2009; Matthijnssens et al., 2008) using a Tm of 50 °C for all the reactions. A 3 min elongation step was used to obtain VP1-4, VP7 and NSP2-5 amplicons, whereas for VP6 and NSP1, elongation was extended to 6 min.

Nucleotide sequencing of amplified genes was performed using the same primers used for RT-PCR in an ABI PRISM® 3130 automated sequencer (Applied BioSystems, Foster City, CA). The Sanger sequencing reaction was based on BigDye Terminator v1.1 chemistry (ThermoFisher Scientific, Waltham, MA).

The sequencing files obtained were analyzed and corrected with Chromas Pro2.23 (Technelysium, Queensland, Australia), and consensus sequences were obtained using SeqMan II (DNASTAR, Madison, WI).

Nucleotide sequence similarity searches were performed using the BLAST server on the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank/index.html). Construction of multiple sequence alignments and phylogenetic trees were performed with the MEGA6 software (www.megasoftwares.com)(Tamura et al., 2013). Sequences of the strains involved in this study were deposited in GenBank, under the following accession numbers: from MH700482 to MH700492.

After nucleotide sequencing, for strain PZ3/2017 a complete genomic constellation 1 (Wa-like) in combination with the G4P[6] genotype was defined: G4-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1. Despite its common Wa-like genomic backbone, this combination has been sporadically detected mainly in Sout-East Asia (Kaneko et al., 2018; Malasao et al., 2018; Nguyen et al., 2007; Zhou et al., 2015), but also in Europe (Papp et al., 2013).

The phylogenetic analysis of VP1 (Fig. 1) revealed the highest nucleotide sequence identities (97.5–98.1 nt id.) between strain PZ3/2017 and RVA strains possessing a R1 VP1 genotype, previously detected in Asia (China, Vietnam) in humans and pigs. Some of these strains possessed a G4P[6] genotype.

The VP2, VP3 and VP6 trees (Fig. 1) showed the same clustering pattern, with the PZ3/2017 included in the same groups of strains possessing a genotype 1, previously detected in Asian countries (China, Vietnam, South Korea, Nepal) from humans and pigs. Italian Wa-like strains included in these trees were grouped separately in different evolutionary clusters.

The phylogenetic tree of VP4 (Fig. 1) defined unambiguously the P[6] genotype for strain PZ3/2017. As for the gene segments mentioned above, the Italian G4P[6] strain clustered together with strains detected in Asia from humans and pigs, mainly in combination with G4 and G9 VP7 genotypes. P[6] strains previously detected in Italy in combination with G3 and G6 VP7 genotypes were grouped separately. Interestingly, the VP4 belonging to the G4P[6] strains detected in Europe (Hungary) showed low nucleotide identity (90.5%) with that possessed by strain PZ3.

The VP7 tree (Fig. 1) defined the G4 lineage VII genotype for strain PZ3/2017, being grouped with G4 strains detected previously in Latin America (Dominican Republic, Mexico, Paraguay, Argentina) and Asia (Philippines, China), from humans and pigs. All the G4 Italian strains in the tree were included in the G4-I lineage, presenting a 85.2% nucleotide identity with strain PZ3/2017. Interestingly, the Chinese strains correlated with strain PZ3/2017 in the VP1-3 and 6 trees were grouped separately, as well as the Hungarian G4P[6] strains, posing the evidence of possible reassortment events at the origin of strain PZ3/2017.

The phylogenetic trees of the NSP genes (Fig. 2) revealed a different clustering for strain PZ3/2017 than that observed for the VPs, showing a mixed pattern of nucleotide sequence similarities. In fact, in the NSP1 tree, strain PZ3/2017 was grouped with G4P[6] strains from Brazil, Ecuador and Hungary. In the NSP2 tree, the highest nucleotide identities were displayed with strains from Argentina and Central Africa (Congo, Uganda), while the NSP3 tree analysis revealed high nucleotide identities with G4P[6] and G3P[6] strains from Asia (Japan, China) and with G9P[23] swine strains identified in Northern Italy in 2009. To the other side the NSP4 and NSP5 trees showed a similar clustering than that observed for the VPs.

The analysis conducted showed that strain PZ3/2017 was always related with a high statistical support to porcine strains or to human strains detected after an inter-species transmission. For this reason, we can conclude a complete swine origin and an inter-species transmission of the G4P[6] RVA strain detected in Southern Italy, excluding a reassortment between human and animal strains.

To the other side, the phylogenetic analyses of the 11 gene segments belonging to strain RVA/Human-wt/ITA/PZ3/2017/G4P[6] showed strong evidences of reassortment events between different porcine viruses at the origin of this RVA strain. The mixed pattern of clustering observed in the trees revealed separate origin of the gene segments from strains detected previously worldwide from pigs and from humans. Human RVAs related to strain PZ3/2017 were all generated from putative inter-species reassortment, even if involving gene segments possessing typical human genotypes shared with the swine host. Due to the unique detection of this strain in the same geographical area and period (data not shown), we hypothesized that these reassortment events could have happened in foreign countries, with the following importation of this uncommon strain from abroad. In addition, there were no detections of other G4P[6] strains following this isolation, indicating a possible dead-end infection of this animal strain in a human host, as already observed (Kaneko et al., 2018).

The sporadic detection of zoonotic RVA strains in the human population highlights the relevance of the constant monitoring of the circulating RVA strains. Moreover, further studies on molecular epidemiology of RVAs in both humans and animals is needed to clarify possible zoonotic and inter-species transmission of uncommon viral variants.

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