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Short communication

Detection of rare reassortant G5P[6] rotavirus, Bulgaria

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ABSTRACT

During the ongoing rotavirus strain surveillance program conducted in Bulgaria, an unusual human rotavirus A (RVA) strain, RVA/Human/BG/BG620/2008/G5P[6], was identified among 2200 genotyped Bulgarian RVAs. This strain showed the following genomic configuration: G5–P[6]–I1–R1–C1–M1–A8–N1–T1–E1–H1. Phylogenetic analysis of the genes encoding the neutralization proteins and backbone genes identified a probable mixture of RVA genes of human and porcine origin. The VP1, VP6 and NSP2 genes were more closely related to typical human rotavirus strains. The remaining eight genes were either closely related to typical porcine and unusual human–porcine reassortant rotavirus strains or were equally distant from reference human and porcine strains. This study is the first to report an unusual rotavirus isolate with G5P[6] genotype in Europe which has most likely emerged from zoonotic transmission. The absence of porcine rotavirus sequence data from this area did not permit to assess if the suspected ancestral zoonotic porcine strain already had human rotavirus genes in its genome when transmitted from pig to human, or, the transmission was coupled or followed by gene reassortment event(s). Because our strain shared no neutralization antigens with rotavirus vaccines used for routine immunization in children, attention is needed to monitor if this G–P combination will be able to emerge in human populations. A better understanding of the ecology of rotavirus zoonoses requires simultaneous monitoring of rotavirus strains in humans and animals.

Group A rotaviruses (*Rotavirus A*, RVA) belong to the genus *Rotavirus* within the family *Reoviridae*. RVs have characteristic morphology consisting of non-enveloped triple-layered icosahedral capsid of 60–75 nm in diameter and a 11-segmented double-stranded RNA genome. The two main viral proteins, VP7 (glycoprotein, G) and VP4 (protease-sensitive protein, P), elicit neutralizing antibodies and segregate independently. Based on this, RVs have been classified into G and P types. So far, at least 27 G types and 35 P types have been detected in avian and mammalian RVAs (Matthijnssens et al., 2011a,b), out of which 12 G types (G1–G6, G8–G12 and G20) and 15 P types (P[1]–P[11], P[14], P[19], P[25], and P[28]) have been detected in human strains. More recently, a classification scheme based on the genetic characterization of all 11 gene segments has been proposed for RVAs with the aim to assist in tracing the origin and evolution of RVAs (Matthijnssens et al., 2008). Different genome segments have been divided into 9 VP1 or R, 9 VP2 or C, 8 VP3 or M, 35 VP4 or P, 16 VP6 or I, 27 VP7 or G, 16 NSP1 or A, 9 NSP2 or N, 12 NSP3 or T, 14 NSP4 or E, and 11 NSP5/6 or H genotypes, in a recent review confirming the great genetic heterogeneity of RVAs (Matthijnssens et al., 2011a).

RVAs are the major cause of acute gastroenteritis in young children and animals. In humans, RVA infections are associated with high morbidity and mortality and significant direct and indirect medical costs. Vaccination is believed to be the only effective measure to control the illness. Two RV vaccines – Rotarix® (GlaxoSmith Kline Biologicals) and Rotateq® (Merck&Sanofi MSD) have been available worldwide since 2006. To evaluate the impact of RV

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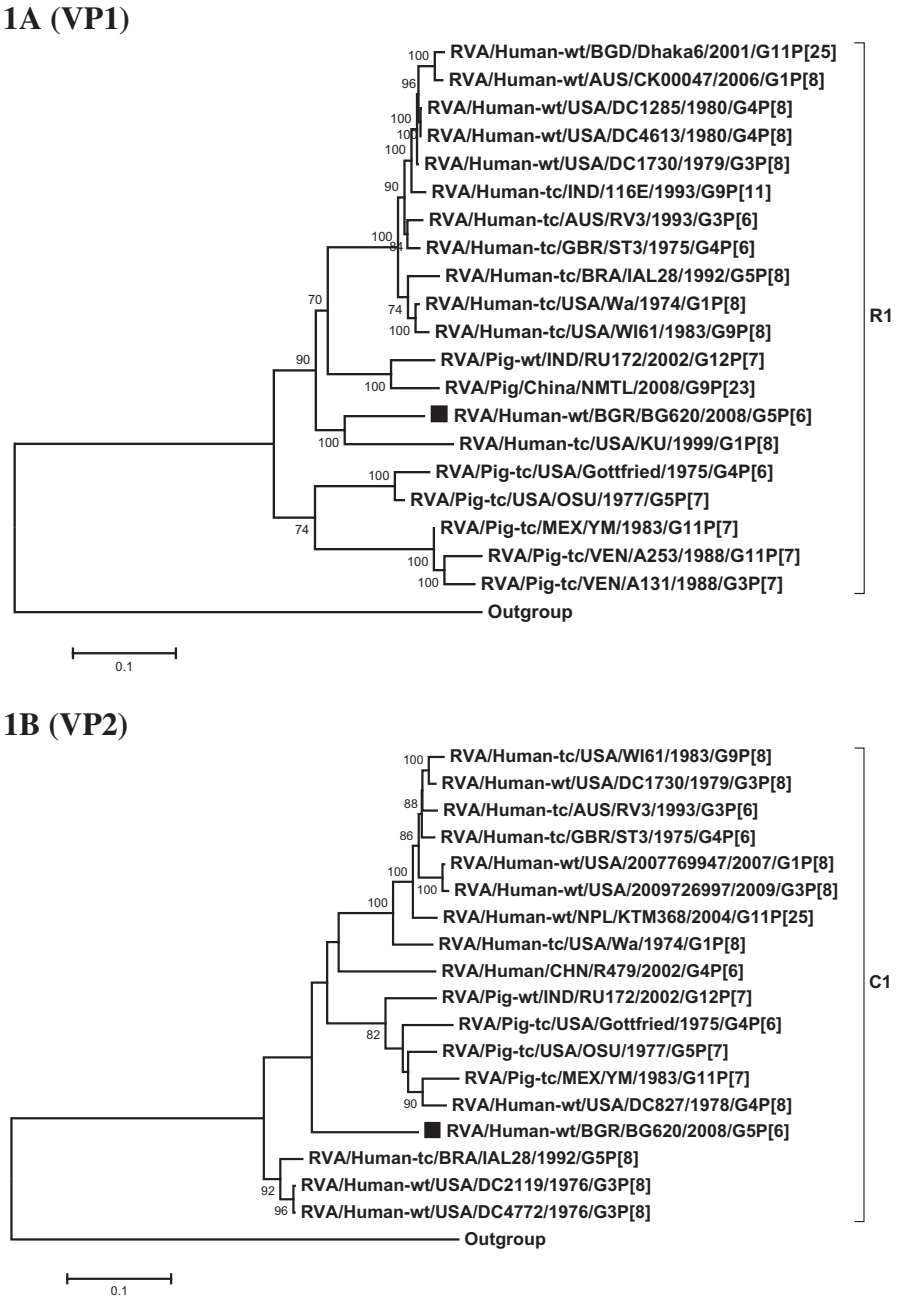

Fig. 1. Phylogenetic trees based on the full-length or partial nucleotide sequences of 11 rotavirus genes coded for viral proteins VP1 (3267 bp), VP2 (2673 bp), VP3 (2230 bp), VP4 (2272 bp), VP6 (1194 bp), and VP7 (981 bp), and nonstructural proteins NSP1 (1461 bp), NSP2 (954 bp), NSP3 (951 bp), NSP4 (620 bp), and NSP5/6 (595 bp). The trees were constructed using the maximum likelihood method from MEGA5 program. Scale bars indicate genetic distance between sequences of segments analyzed. Bootstrap values over 70% using 500 replicates are shown. The Bulgarian strain RVA/Human/BG/BG620/2008/G5P[6] is marked with symbol ■. Different genotypes of the 11 rotavirus genome segments are noted in the right-hand side in square brackets.

vaccine on the disease burden, epidemiology, strain diversity and virus ecology, a number of national and international RVA surveillance networks have been established. EuroRotaNet is an European collaborative network encompassing 18 participating countries (including Bulgaria), established with the aim to perform RVA strain surveillance in Europe (Iturriza-Gómara et al., 2011).

In anticipation to the introduction of RV vaccines, the Bulgarian Rotavirus Surveillance Network was launched in 2005 and during the subsequent seven years a total of 2200 RVA strains were genotyped (Mladenova et al., 2010, in press). Sixty-nine RV strains remained G and/or P untypeable using previously published genotyping RT–PCR assays (Gouvea et al., 1990; Gentsch et al., 1992; Iturriza-Gómara et al., 2000, 2004; Simmonds et al., 2008) and 23 of these strains were randomly selected for nucleotide sequencing of the VP7 and VP4 genome segments. Of interest, a rare G–P combination, G5P[6], was identified in the stool sample collected from a 30-month old girl in July 2008 (RVA/Human-wt/BGR/BG620/2008/G5P[6]; short name, BG620). The child was a resident of a small village in rural area near Sofia and was hospitalized with symptoms of acute gastroenteritis: vomiting (2–3 episodes/day), acute watery diarrhea (4–6 episodes/day for 3 days) and moderate dehydration. Although the G5 VP7 specificity combined with either P[6], P[7] or P[8] VP4 genotype has been found in children in South America, Africa and parts of Asia (Leite et al., 1996;