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# Rotavirus genotypes in Belarus, 2008–2012 ☆

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ABSTRACT

This study describes group A rotavirus (RVA) genotype prevalence in Belarus from 2008 to 2012. In 2008, data from 3 sites in Belarus (Brest, Mogilev, Minsk) indicated that G4P[8] was the predominant genotype. Data from Minsk (2008–2012) showed that G4P[8] was the predominant RVA genotype in all years except in 2011 when G3P[8] was most frequently detected. Other RVA genotypes common in Europe (G1P[8], G2P[4]) were detected each year of the study. This study reveals the dominance of genotype G4P[8] in Belarus and helps to establish the baseline genotype prevalence prior to RVA vaccine introduction in the country.

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## 1. Introduction

Group A rotavirus (RVA) is a well-known etiological agent of acute gastroenteritis that mostly affects small children. The virus is widespread and plays a dominant role among causative agents of acute gastroenteritis both in developed and developing countries. It is believed that every child has an RVA infection at least once before 5 years of age. Moreover, RVA often causes a severe form of gastroenteritis (RVGE) with severe dehydration that often requires oral or intravenous rehydration (Sack et al., 1978). Inadequate therapy can lead to a lethal outcome which is a serious problem for developing countries and is estimated to result in 453,000 deaths annually prior to the introduction of vaccination (Tate et al., 2012).

RVA is a nonenveloped virus that belongs to the *Reoviridae* family. The virus genome contains 11 segments of double-stranded RNA that code 6 structural and 6 nonstructural proteins (Estes and Kapikian, 2007). RVA have two independently segregating serotype antigens, outer capsid proteins VP4 (P type) and VP7 (G type) coded by segments 4 and 9, respectively (Estes and Kapikian, 2007). There are at least 27 different G genotypes and 35 P genotypes known today (Matthijnssens et al., 2011). Among at least 12 G types and 15 P types identified in humans, more than 70 G–P antigen combinations have been detected (Matthijnssens et al., 2009).

Although RVA genotypes vary from year to year in particular regions, 5 common G/P combinations have been identified in Europe: G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] (Iturriza-Gomara et al., 2009, 2011; Usonis et al., 2012). In the last 2 decades genotype G1P[8] has been predominant, being responsible for about 70% of RVA gastroenteritis in Europe (Santos and Hoshino, 2005). Recently, G9P[8] emerged in different parts of the world and now represents a globally common strain that is apparently becoming more widespread over time and it has been suggested that genotype G12 is also emerging to potentially become another globally important strain (Matthijnssens et al., 2009, 2010).

Development of new RVA vaccines and their recent licensing in many countries has resulted in the inclusion of RVGE as another vaccine preventable disease. Consequently, surveillance studies are needed in countries considering the use of RVA vaccines to monitor strain prevalence. In the Belarus laboratory, diagnosis of RVA infection by using enzyme immunoassays (commercial kits as well those produced in the Republican Research and Practical Center for Epidemiology and Microbiology) has been conducted

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for about 15 years but information concerning genotypic diversity of circulating viruses in Belarus is still very limited with only 4 reports published in Russian (Gudkov et al., 2008, 2010, 2011; Samoilovich et al., 2013). The purpose of this study was to determine circulating RVA genotypes in Belarus during 2008–2012. This information will help establish the impact of future RVA vaccination programs in the country.

## 2. Materials and methods

All specimens were convenience samples from children admitted to infectious disease hospitals with a diagnosis of acute RVGE. The stool samples were tested first using a solid phase enzyme immunoassay (EIA for rotavirus antigen revealing, Republican Research and Practical Center for Epidemiology and Microbiology, Belarus). Specimens for genotyping were selected randomly from EIA-positive samples and total of 633 stool specimens were characterized. In 2008, specimens were collected in three regions of Belarus: 115 specimens from the capital Minsk-City (central part of the country), 16 specimens from Brest region (western part of the country), and 13 specimens from the Mogilev region (eastern part of the country). From 2009 to 2012, all specimens were from Minsk with 297 samples analyzed in 2009, 59 in 2010, 68 in 2011, and 65 in 2012.

Viral RNA was extracted from 10% stool suspensions prepared in PBS using a KingFisher Extraction system (Thermo Electron Corp, Finland). G (VP7) and P (VP4) typing were carried out by reverse transcription-polymerase chain reaction (RT-PCR) genotyping as described previously (Hull et al., 2011). Genotype determination of non-typeable samples was performed by nucleotide sequencing as described previously (Hull et al., 2011).

## 3. Results

Of the 633 specimens, G types could be assigned to 628 samples and 5 were G non-typeable (NT). P types were determined for 629 specimens. The predominant G types detected were G1, G2, G3, and G4. Among them G4 type was predominant (336 strains, 53.5%) followed by G2 (105 strains, 16.7%), G3 (101 strains, 16.1%) and G1 (72 strains, 11.5%). Genotypes G9 (5 strains, 0.8%), and G8 (2 strains, 0.3%) were less frequently detected. Among the VP4 specificities, genotype P[8] was most frequent and represented by 80.9% (509 strains) and P[4] was detected in 106 samples (16.9%). Genotypes P[6] and P[9] were present at rates of 1.3 and 0.8%, respectively. The most common G–P combination among the 624 G and P-typed samples was G4P[8] accounting for 52.4% (327) of the pediatric gastroenteritis cases followed by G2P[4] (103 cases, 16.5%) and G3P[8] (96, 15.4%). Genotype G1P[8] was detected in 11.7% of samples (72 cases). Other combinations (G2P[6], G4P[6], G3P[9], G8P[4], G9P[8]) were rare and found only in 1–5 cases each during the study period.

### 3.1. RVA genotype distribution in different regions of Belarus

In 2008, specimens were collected in 3 regions of Belarus, permitting a comparison of genotype prevalence in different parts of the country (Table 1 and Fig. 1). Genotype G4P[8] was predominant in each of three locations studied: Brest region – 81.3% (13 cases), Mogilev region – 53.8% (7 cases), Minsk – 58.3% 9 (67 cases). In the Brest region, the other genotype detected was G1P[8] (3 of 16 cases; Fig. 1). In the Mogilev region, aside from the aforementioned G4P[8], 2 other genotypes were detected, G3P[8] and G1P[8], along with 2 partially-typed strains (Fig. 1). In Minsk, 7 genotypes were detected (Fig. 1). Along with G4P[8] strains, G1P[8] and G3P[8] were present at notable levels (21.7% and 7.8%, respectively; Fig. 1). Genotypes G2P[4], G2P[6], G4P[6], and G9P[8] were also detected at low levels along with some G or P non-typeable strains (Fig. 1). Although the data obtained demonstrate some differences in genotype distribution in 3 regions, the small number of samples tested makes it difficult to ascribe significance to the noted variation in genotypes.

**Table 1**
Distribution of RVA G and P types in Belarus, 2008.

| | P[4] | P[6] | P[8] | NT | Total no. |
|---|---|---|---|---|---|
| G1 | | | 29 | | 29 |
| G2 | 3 | 2 | | | 5 |
| G3 | | | 12 | | 12 |
| G4 | | 4 | 87 | 4 | 95 |
| G9 | | | 1 | | 1 |
| NT | | 1 | 1 | | 2 |
| Total no. | 3 | 7 | 130 | 4 | 144 |

### 3.2. Temporal variation in RVA genotype distribution-Minsk

The 2008 data for Minsk are described in the previous section. In 2009, 297 samples from Minsk were genotyped and 59–68 samples were genotyped in years 2010, 2011, and 2012 (Tables 2–5, Fig. 2). G4P[8] was the predominant genotype in 2008, 2009, 2010, and 2012 with 42.4–58.3% prevalence during these years and it was the second most common genotype in 2011 (23.5%; Fig. 2). In 2011, G3P[8] was the predominant genotype (64.7%) and it was the second most frequently detected genotype in 2010 (27.1%) and 2012 (21.5%; Fig. 2). G2P[4] reached 30.0% prevalence in 2009 and 10.2% in 2010 but was detected less frequently in other years (Fig. 2). The highest detection frequency for G1P[8] was 21.7% in 2008 and its prevalence ranged from 4.4% to 12.3% in 2009–2012 (Fig. 2). Other genotypes (G8P[4], G9P[8], G3P[9]), as well as G or P non-typeable strains, were detected at low frequencies in multiple years (Tables 2–5, Fig. 2). Rare P[6] RVA strains were detected in 2008 (G2P[6], 2 cases; G4P[6], 4 cases) and 2009 (G4P[6], 1 case) but not in other years (Tables 1 and 2, Fig. 2). Six mixed genotype infections were detected in 2009 and 1 was detected in 2011 (Tables 2 and 4, Fig. 2).

## 4. Discussion

Worldwide RVA surveillance has permitted establishment of the most common genotypes for different geographic areas (Banyai et al., 2012). At the same time, current studies are being carried out in many countries that have not previously been surveyed, providing new data and constantly updating our knowledge of the prevalence and epidemiological significance of individual RVA genotypes.

Investigation of clinical samples from patients with acute RVGE in Belarus from 2008 to 2012 revealed high genetic diversity in circulating RVA strains. It was confirmed that RVA of all the most predominant genotypes from Europe circulate in Belarus, namely G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]. These genotypes were responsible for 94.8% (600/633) of RVA cases countrywide. In addition, some rare genotypes (i.e., G4P[6], G8P[4], G3P[9]) were detected in multiple study years. Genotype G4P[8] was the predominant etiological agent of RVGE in Belarus during the time of investigation, which was responsible for about 52% of cases in the country and confirms finding of previous studies in Belarus (Gudkov et al., 2008, 2010, 2011; Samoilovich et al., 2013). This is in contrast to results of contemporary studies conducted in other European countries which demonstrated that genotype G1P[8] was the predominant strain in the region in most years (Laszlo et al., 2012; Ruggeri et al., 2011; Vesikari et al., 2013). However,