NOROVIRUS INFECTION IN BELARUS: OCCURRENCE AND MOLECULAR EPIDEMIOLOGY

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SUMMARY

The objective of the study is to analyze molecular epidemiologic surveillance for norovirus infection in Belarus over the past five years (2009–2013). Laboratory diagnostics was carried out by RT-PCR in 684 patients. Two regions of norovirus genome, localized in RNA-polymerase and capsid protein genes, were used for phylogenetic analysis.

Noroviruses were predominant causative agents in adults and second only to rotaviruses in children, they also prevailed among aetiological agents of outbreaks (66.7% of outbreaks). In 2009–2013, the major norovirus genotype was GII.4 (58.3% of all genotyped isolates). Genovariant GII.4 2006b circulated in 2009 and 2010, genovariant GII.4 2009 New Orleans – in 2010 and 2012. In addition to GII.4, genotypes GII.6 (16.6%), GII.2 (4.1%), GII.3 (2.2%), and recombinant genotypes GII.g-GII.12 and GII.g-GII.1 (10.4% and 8.3%, respectively) circulated in Belarus.

The findings indicate a significant contribution of noroviruses in development of sporadic morbidity and outbreaks of acute gastroenteritis in Belarus. Outbreaks or prominent increases of sporadic morbidity were mostly due to the emergence of a new genotype, or an epidemic genovariant.

Key words: norovirus, acute gastroenteritis, group and sporadic morbidity, genotype

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INTRODUCTION

Norovirus (NoV) is a genus of small non-enveloped viruses with (+)ssRNA genome in the Caliciviridae family. The genus comprises 5 distinct virus genogroups, 3 of which (I, II, and IV) are human pathogens (1). In the mid 1990's, a significant increase of NoV infection incidence was registered worldwide; the reasons for such elevation in virus activity are not fully understood. At the present time, noroviruses are the most common cause of acute gastroenteritis in adults and dominant aetiological agents of acute enteric infections outbreaks, including foodborne infections (2, 3). In paediatric patients noroviruses are also common cause of acute gastroenteritis second only to rotaviruses (4).

Noroviruses are characterized by extremely high level of genetic variability underlying their genetic and phenotypic diversity (5). The latter factor accounts for rapid emergence and concurrent circulation of multiple antigenic variants, which, on the one hand, prevents formation of sustainable immune response in the course of natural immunization, and, on the other hand, hinders development of effective vaccines (6). Due to lack of specific means for treatment and prevention of NoV infection, the most productive current strategy to restrain transmission of the disease involves regular epidemiological surveillance for the circulation of viral agents, based on the results of laboratory control procedures and molecular-epidemiological investigations. Such surveillance permits to detect in a timely manner the emergence and dissemination of the new epidemic genovariants capable of eliciting significant rise of morbidity and to take opportune anti-epidemic measures.

In Belarus, routine laboratory diagnostics and research into molecular epidemiology of NoV infection, including genotyping of its agents, have been under way since 2009. Concurrently, investigations of aetiologic structure of acute enteric infection and gastroenteritis outbreaks, caused by adeno-, astro-, and sapoviruses, have been carried out.

The present paper summarizes data collected in Belarus over the past 5 years (2009–2013) in the scope of molecular-epidemiological surveillance for NoV infection agents.

MATERIALS AND METHODS

Stool samples were collected from 684 patients, including 577 children (397 children younger than 5 years, 180 children aged 5–18 years) and 107 adults within 24–48 hours of onset of the disease. All patients were hospitalized during 2009–2013 with diagnosis of acute gastroenteritis (n = 496), acute enteric infection (n = 88), food toxicoinfection (n = 31), acute enteritis (n = 44), acute enterocolitis (n = 17), acute gastritis (n = 4), and hemocolitis (n = 4).

Commercial kits RIBO-SORB and RIBOPREP (AmpliSens, Russia) were used to isolate RNA from clinical samples in accordance with manufacturer's directions. Reverse transcription was carried out with RevertAid kit (Fermentas, Lithuania), or REVERTA kit (AmpliSens, Russia).

Differential laboratory diagnostics was performed with the help of commercial kits Rotavirus/Norovirus/Astrovirus-FL, AEI screen-FL, and Norovirus 1,2 genotypes-EPh (AmpliSens®, Russia), as well as with multiple primer sets described elsewhere (7–9).

Two fragments of norovirus genome were selected for sequencing: a 340 nt fragment in 3' RdRp region, and a 280 nt portion of VP1 gene (7, 8). DNA sequencing was performed by chain termination in thermocyclic reaction with the help of DTCS Quick Start Kit and automatic DNA-analyzer CEQ8000 (Beckman Coulter).

BLAST (Basic Local Alignment Search Tool) was used for homologous sequences search in the NCBI database (10). Computer sequence analysis (multiple alignment, calculation of evolutionary distances, phylogenetic reconstruction, and testing of tree topology) was performed with MEGA (Molecular Evolutionary Genetics Analysis) software package, version 6.0 (11). Maximum likelihood method and corrected Akaike information criterion (AICc) were employed to determine optimal mathematic model of nucleotide substitution. Phylogenetic reconstruction was based on neighbour joining (NJ) and maximum likelihood (ML) algorithms implemented in MEGA 6.0. Bootstrap analysis was used for testing of the phylogenetic trees topology with 1000 pseudoreplicates analyzed for each tree (12).

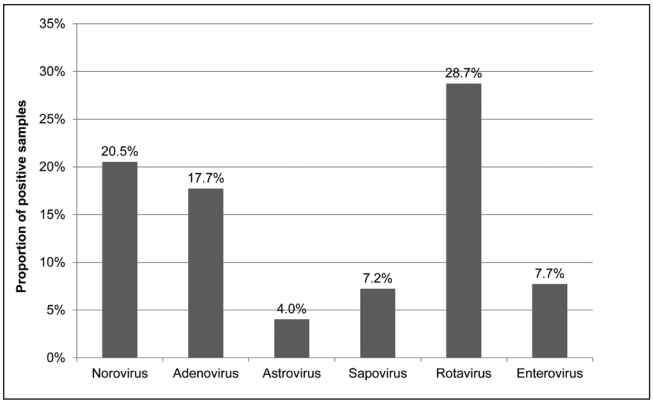

Fig. 1. *NoV share in the aetiology of viral acute enteric infections.*

RESULTS

Results of laboratory diagnostics performed in the setting of sporadic morbidity for selected patients with clinical diagnosis of acute enteric infection in the years of 2009–2013 as well as data on the aetiology of outbreaks suggest considerable share of noroviruses in the structure of viral enteric infections. In general, noroviruses were second only to rotaviruses in terms of detection rate (Fig. 1). They were predominant causative agents of acute enteric infections in adults: 30.0% of acute gastroenteritis cases were caused by norovirus, whereas only 8.6% by rotavirus. Genogroup II NoV were prevalent aetiologic factors of intestinal disease group morbidity and caused 60.0% of outbreaks, whereas Sapovirus only 13.3%, Salmonella spp. 6.7 %, Rotavirus 6.7%, Norovirus I 6.7%, and Enterovirus 6,7% of registered episodes. Detection rate of NoV from genogroup II had clear seasonal variation: proportion of sporadic morbidity associated with NoV GII was 3 times higher in winter than in summer (29.6% vs 10.6%, respectively). However, occurrence of NoV infection group morbidity was almost on the same level all year round.

From 2009–2013, a total of 15 group morbidity episodes registered in different seasons were investigated to establish the underlying aetiology (Table 1). One hundred and four patients

Table 1. *General characteristics of acute enteric infections outbreaks in Belarus, 2009–2014*

Data (month year)	Region	Clinical diagnosis	Number of assessed patients	Age	Detected agent(s)	Number of patients with established aetiology
May 2009	Minsk	AGE	8	18–20	Sapovirus	6
February 2010	Minsk region	AGE	10	18–20	Sapovirus	6
					Norovirus II	1
					Enterovirus	3
March 2010	Minsk	AGE	8	45–86	Norovirus II	7
May 2010	Minsk	AGE	15	16–17	Norovirus II	13
February 2011	Minsk	AGE	5	18–25	Norovirus II	4
					Enterovirus	2
January 2012	Lyahovichi	AGE	11	11–18	Norovirus II	9
May 2012	Luninets	FTI	5	7–8	Norovirus II	5
July 2012	Borisov	AGE	6	7–12	Enterovirus	6
July 2012	Myadel	AGE	5	9–15	Norovirus I	4
July 2012	Minsk	AGE	6	18–20	Norovirus II	2
October 2012	Luninets	AGE	3	16–17	Norovirus II	2
July 2013	Minsk region	AGE	7	6–13	Norovirus II	6
					Enterovirus	1
August 2013	Minsk region	AGE	6	18–20	Salmonella spp.	5
October 2013	Minsk region	AGE	6	18–20	Rotavirus A	5
January 2014	Minsk region	AGE	2	10–12	Norovirus II	2

Abbreviations: AGE – acute gastroenteritis; FTI – food toxicoinfection.

were assessed in regard of viral (Rotavirus, Norovirus GI and GII, Astrovirus, Adenovirus, Sapovirus, and Enterovirus), or bacterial (Salmonella, Shigella and Campylobacter) intestinal infections. Almost all of the reported episodes occurred in closed or semi-closed communities, such as children's summer camps, boarding schools, military bases, etc. Age of the patients ranged from 6 to 86 years.

Primary clinical diagnosis was acute gastroenteritis. The majority of group morbidity episodes were caused by a single infectious agent (73.3%, positive 11/total 15), while 4 of them were associated with concomitant transmission of several (2–3) viruses. In such cases the pathogen revealed in more than one third of examined patients was considered the aetiologic agent of the outbreak. The data indicate dominant role of NoV as the cause of acute enteric infections group morbidity: 66.7% of episodes were caused by NoV from genogroups I and II, comprising 60% of members of genogroup II and 6.7% of members of genogroup I. In half of the reported episodes noroviruses were the only aetiologic agents identified.

Genotyping of NoV isolates and molecular-epidemiological surveillance for NoV infection were based on sequence analysis of two virus genome fragments. In total, 48 NoV isolates from patients of different age were assessed. The viruses were collected during periods of sporadic morbidity and at the time of outbreaks. Two genomic regions were selected for molecular-epidemiological investigations: a fragment of major capsid protein VP1 gene and a fragment of RNA-dependent RNA-polymerase gene, which allow molecular typing and identification of known epidemic genovariants of NoV. For 16 virus isolates analysis was performed in parallel on 2 genomic regions, this provided opportunity to identify recombinant genovariants. Analysis of 38 isolates was based on RNA-polymerase gene fragment only, and for 25 isolates a portion of VP1 gene was used. Neighbour joining and maximum likelihood phylogenetic trees derived from sequence analysis of the two genomic regions are presented in Figures 2 and 3.

The results showed that 58.3% of NoV isolates (28/48) belonged to genotype GII.4, currently the most prevalent NoV genotype worldwide. Within genotype GII.4 two global genovariants were identified: 33.3% of NoV isolates (16/48) belonged to genovariant GII.4 2006b, whereas 25.0% of NoV isolates (12/48) were identified as members of genovariant GII.4 2009 New Orleans. In addition to the major genotype GII.4, other NoV genotypes circulated in Belarus: GII.6 (16.6%), recombinant genotypes GII.g-GII.12 and GII.g-GII.1 (10.4% and 8.3%, respectively), GII.2 (4.1%), and GII.3 (2.2%).

DISCUSSION

Analysis of the presented NoV phylogenetic reconstruction allows us to describe molecular epidemiology of NoV in Belarus between 2009 and 2013. As can be expected, the majority of genotyped NoV isolates belonged to the most prevalent GII.4 NoV genotype. To date, within this genotype 6 distinct genovariants with global circulation have been identified, which accounted for

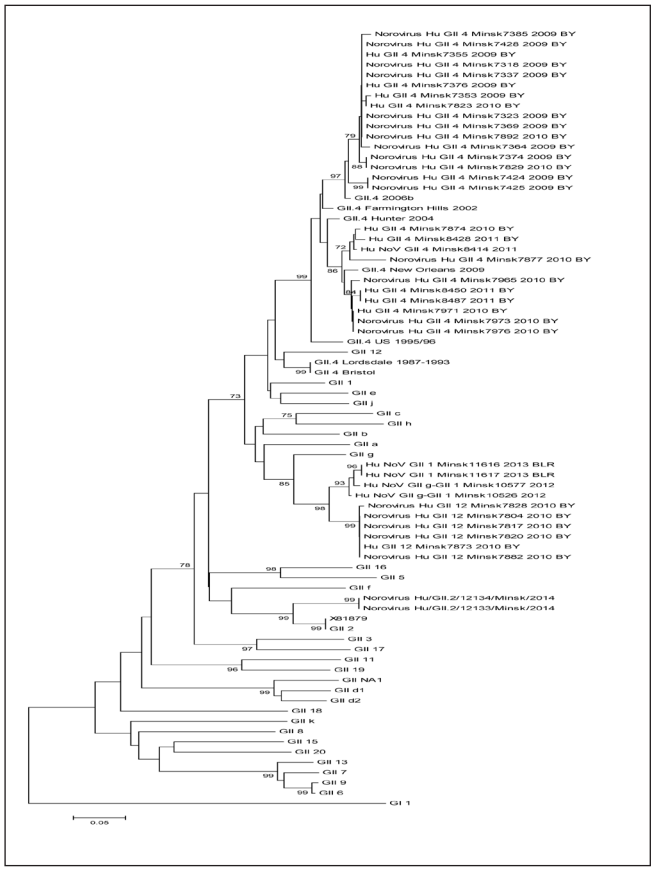

Fig. 2. *NJ phylogenetic tree based on nucleotide sequence analysis of RNA-polymerase gene fragment of 38 NoV isolates from patients with acute enteric infection (Belarus, 2009–2014).*

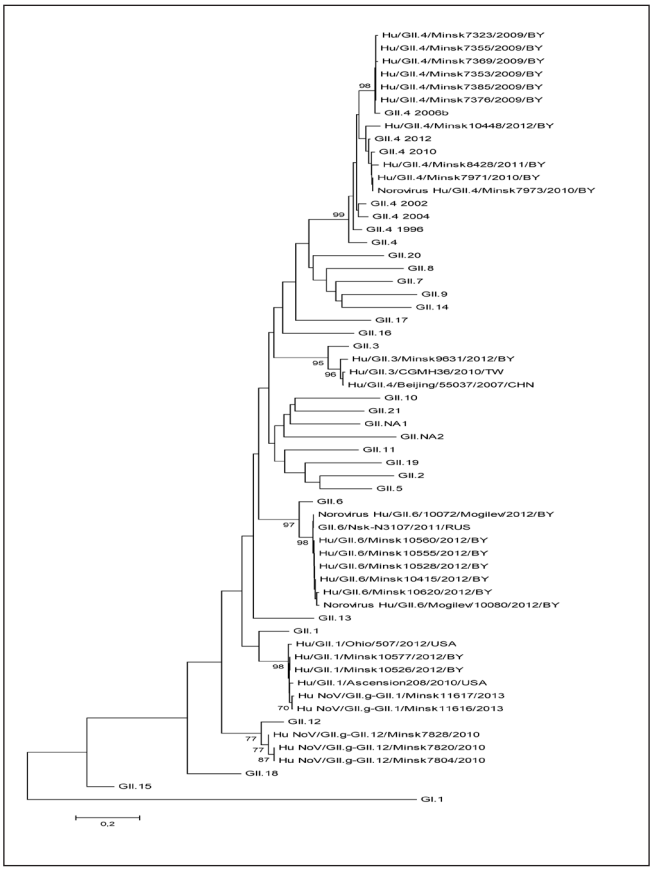

Fig. 3. *ML phylogenetic tree based on nucleotide sequence analysis of 280 nt VP1 gene fragment of 25 NoV isolates from patients with acute enteric infection (Belarus, 2009–2013).*

62–80% of NoV infection outbreaks in different countries (13, 14). These genovariants include US 1995/96 (1996), Farmington Hills (2002), Hunter (2004), 2006b (2007–2008), New Orleans (2009–2012), and Sydney (2012–2014) strains. Phylogenetic analysis of Belarusian NoV isolates revealed local circulation of 2 out of 6 global genovariants belonging to genotype GII.4: sporadic morbidity of 2009–2010 was caused by genovariant GII.4 2006b, while the causative agent of 3 episodes of group morbidity (March and May 2010, and January 2011) was identified as a member of genovariant GII.4 2009 New Orleans. In 2011–2012, circulation of the latter genovariant continued and NoV GII.4 2009 New Orleans isolates were detected in patients with sporadic cases of NoV infection.

Genotype GII.3 was represented by a single agent that caused an episode of group morbidity during winter 2012; that was the only instance when circulation of this strain was registered in the country. Similarly, genotype GII.2 caused a single episode of group morbidity in children in 2014, but was never detected in the setting of sporadic morbidity. Genotype GII.6 was the culprit in two group morbidity episodes – in July and October 2012 – and was also identified in sporadic cases of NoV infection. Genotype GII.g-GII.12 for the first time emerged in Belarus in 2010 together with epidemic genovariant GII.4 New Orleans caused significant increase of sporadic NoV infection incidence during winter 2011 (the proportion of NoV-associated acute enteric infections was 1.9–1.4 times higher in winter 2011 than in 2009–2010, data not showed). Recombinant genotype GII.g-GII.1 was registered in sporadic cases of NoV infection in summer 2012 and later, in July 2013, caused an episode of group morbidity in children.

Of note, outbreaks and seasonal increases of morbidity caused by recombinant NoV genotypes GII.g-GII.12 and GII.g-GII.1 were almost simultaneously observed in different countries worldwide. Thus, in 2010, genotype GII.g-GII.12 was the causative agent of winter increase of morbidity in USA (16% of all reported outbreaks) (15), while genotype GII.g-GII.1 was prevalent in Belgium and caused a major foodborne infection outbreak in a German hospital in 2012 (16).

CONCLUSION

In summary, it is evident that over the past 5 years significant level of genetic diversity and rapid succession of circulating NoV genotypes and genovariants were observed in Belarus. The new epidemic genovariants emerged almost at the same time, these agents spread in other parts of the world and generally were accompanied by increases of morbidity, group or sporadic. These findings support the necessity for regular molecular-epidemiological surveillance for NoV infection which will allow early detection of new virus genovariants and prognostic assessment of their epidemic potential.

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