

Figure. Locations where isolates of African swine fever virus were obtained in Russia during or after 2011. Black circles indicate isolates with tandem repeat insertions, and white circles indicate isolates without tandem repeat insertions. obl., oblast; Resp., respublika.

Spatial and temporal patterns were evaluated by using a map generated with the ArcMap package implemented in ArcGIS software 10.1 (ESRI CIS Ltd., Moscow, Russia). This map shows geographic locations of virus isolates from 2011 onwards (Figure). The alignment, as well as the geographic distribution of the available isolates, clearly shows that the TRS insertion was present in 2012, especially in the Russian Tulsckaya oblast (Figure). The TRS insertion predominates in subsequent isolates, although isolates without the TRS insertion are still present. Furthermore, this TRS insertion was also present in Ukraine in 2012 but could not be found in any isolates obtained in the Tverskaya Oblast in 2011 and 2012 (Figure).

In conclusion, these findings confirm the suitability of the described TRS for a higher resolution of ASFV molecular epidemiology. However, this TRS insertion was already present in ASFV strains from Russia and is not restricted only to strains from central Europe. Thus, it can be hypothesized that viruses introduced into the European Union originated in Russia, emerged in 2012 or even earlier, and were transmitted through Belarus and Ukraine.

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Norovirus GII.21 in Children with Diarrhea, Bhutan

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To the Editor: Noroviruses are nonenveloped viruses of the family *Caliciviridae* with a single-stranded RNA genome. In developing countries, noroviruses cause >200,000 deaths annually among children <5 years of age (1). Noroviruses are divided into 6 genogroups, GI–GVI, which are further divided into genotypes. Twenty-nine of the genotypes cause human infections (2). Worldwide, most GII infections are caused by GII.4, followed by GII.3 or GII.6 and then other genotypes, such as GII.2, GII.12, GII.13, GII.17, and GII.7, in varying proportions. We report that GII.21 is the major genotype causing diarrhea in children in Bhutan.

During February 2010–December 2012, fecal samples were collected from children <5 years of age with watery diarrhea who were seen at the outpatient and inpatient clinics of the Department of Pediatrics, Jigme Dorji Wangchuk National Referral Hospital, Thimphu, Bhutan. We extracted RNA from rotavirus-negative fecal samples by using the QIAamp Viral RNA Kit (QIAGEN, Hilden, Germany); norovirus was detected by reverse transcription PCR by amplifying capsid gene at C region (3). PCR results were confirmed by nucleotide sequencing of the amplicons (4). We determined genogroups and genotypes by submitting nucleotide sequences to the Norovirus Genotyping Tool (<http://www.rivm.nl/mpf/norovirus/typingtool>).

We collected 15 water samples (online Technical Appendix Table, <http://wwwnc.cdc.gov/EID/article/21/4/14-1856-Techapp1.pdf>), including some from streams and water tanks that are sources of the Thimphu city water supply. Some samples were arbitrarily collected from taps from different locations in Thimphu. All samples were assayed for total coliforms, thermotolerant coliforms, and norovirus (5).

We performed a multiple sequence alignment using MUSCLE and conducted the phylogenetic analyses with the neighbor-joining method using MEGAS software (<http://www.megasoftware.net>). The branching patterns were evaluated statistically on the basis of bootstrap analyses of 1,000 replicates.

We tested 270 samples for norovirus. The mean age of children tested was 13.9 months. Sixty-four (23.7%) children were positive for norovirus. Results were positive for genotype GI in 4/210 samples (0/56 in 2010, 3/123 in 2011, and 1/31 in 2012); all positive samples were from boys in the outpatient clinic. Genotype distribution of norovirus GI was 1 GI.3 in 2012 and 1 GI.1 and 2 GI.9 in 2011.

Sixty of the 270 samples were positive for genotype GII norovirus (17/73 in 2010, 24/147 in 2011, and 19/50 in 2012). Of these, 32 (53.3%) were from boys. Forty-six children were outpatients. Most (85%) of the GII norovirus cases occurred in children <23 months of age; 63.3% of infection occurred in children 6–23 months of age (online Technical Appendix Figure 1). By patient age group, the pattern for norovirus GII.21 was similar; 76% of infections occurred in children <23 months, and 62% infections occurred in children 6–23 months.

We determined seasonal distribution of norovirus GII for 52 cases; 21 cases occurred during winter, and only 2 cases occurred during autumn. Nine cases each occurred during spring and summer, and 11 cases occurred during the rainy season (late June through late September). In 2010, GII.3 predominated, followed by GII.4 and GII.7; in 2011, GII.21 was dominant, followed by GII.6, GII.2, and GII.8, then others; and in 2012, GII.21 remained the dominant strain, followed by GII.2, GII.4, and GII.6 (Figure).

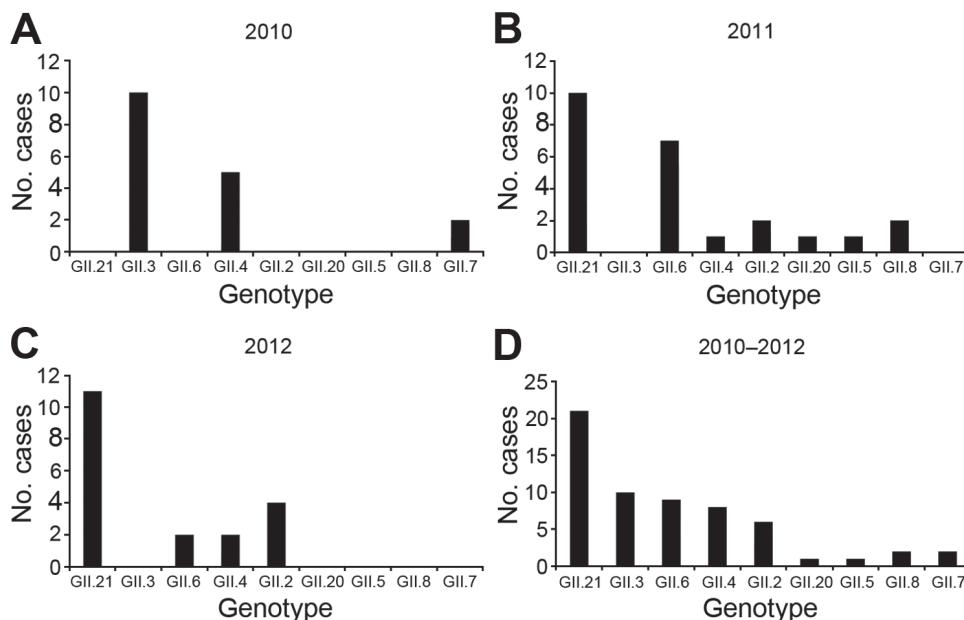


Figure. Yearly distributions of different genotypes of norovirus genogroup GII (A–C) and total numbers (D), Bhutan.

A Sydney variant of norovirus GII.4 was identified in 2012. Only 1 tap water sample (sample W8) from a house was positive for norovirus and coliform bacteria; and nucleotide sequencing of the norovirus amplicon confirmed it as GII.21. Phylogenetic analysis showed that non-GII.21 genotypes from Bhutan were closely associated with strains from Thailand and Korea (online Technical Appendix Figure 2).

In Bhutan, similar to other countries, genogroup GII is mainly responsible for norovirus infections, and most infections occur in younger children (6,7). In 2010, norovirus GII.3 was the dominant genotype in Thimphu, but in 2011, GII.21 became dominant and continued throughout 2012. GII.21 has been identified mostly in wastewater or rivers, and infections in human have been infrequently attributed to it (8). One GII.21 outbreak in a long-term care facility for elderly persons has been reported from the United States (9).

Why children in Thimphu were infected by GII.21 is not clear. The detection of norovirus GII.21 in 1 water sample suggests that the source of the outbreak might be tap water; however, GII.21 was not detected in the water related to the tap water supply system. Further examination using repeated samples from different sources is needed. Continuous dominance by GII.21 over 2 years indicates that norovirus of this genotype might have been established in the children of Thimphu, and human-to-human transmission might be ongoing. Determining the environmental source of norovirus GII.21 in Bhutan and developing prevention strategies to control the spread are urgently needed.

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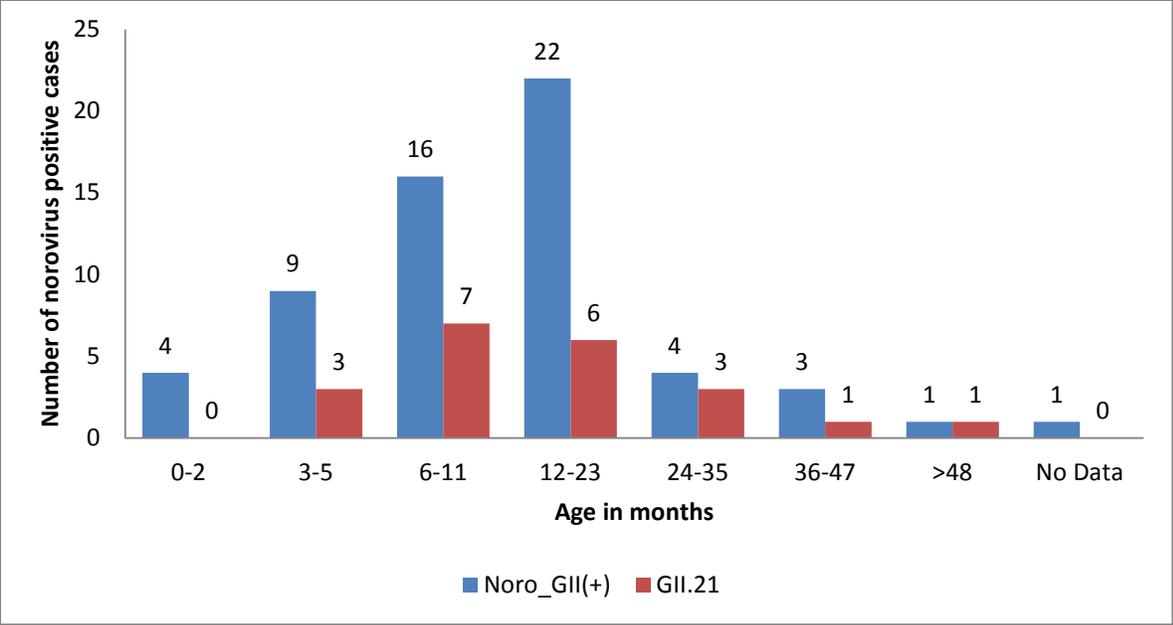
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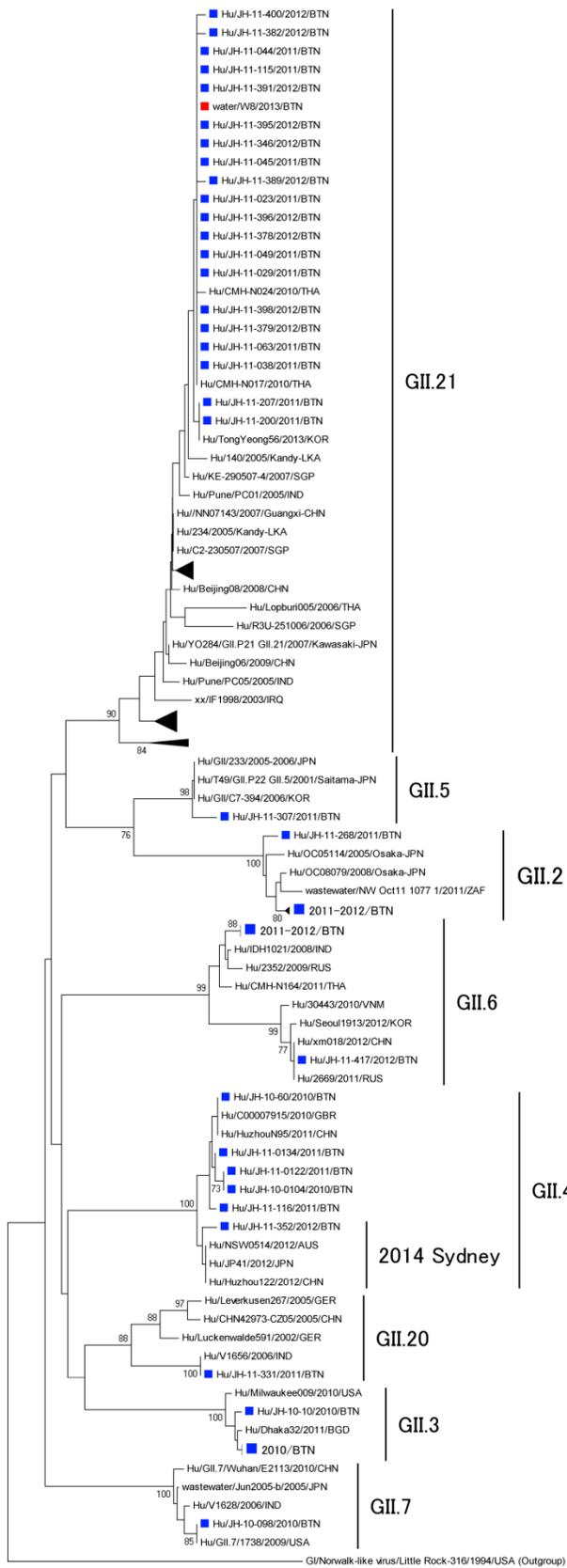
Technical Appendix

Technical Appendix Table. Sample collection date and detailed information of the location, temperature, pH, and fecal coliform count of the samples

Sample no.	Collection date	Place	Longitude	Latitude	Elevation, m	Water temperature, °C	pH	Fecal CFU/100 mL
A	2013/11/21	Motithang source (Big stream)	27°28'43.5"	89°35'48.6"	2800	4.5	8.1	None
B	2013/11/21	Motithang source (Small stream)	27°28'43.7"	89°35'48.5"	2803	5	7.5	3
W1	2013/11/22	Motithang source (Big stream)	27°28'43.5"	89°35'48.6"	2800	4	8.2	None
W2	2013/11/22	Motithang source (Small stream)	27°28'43.7"	89°35'48.5"	2803	5.5	7.8	None
W3	2013/11/22	Junshina source	27°30'14.0"	89°38'08.0"	2343	5	8.2	None
W4	2013/11/22	Cheri side	27°35'36.4"	89°37'42.9"	2574	4.5	8.7	None
W5	2013/11/22	Dodena river	27°35'36.0"	89°37'44.3"	2574	4.5	8.6	None
W6	2013/11/22	Wangchu river	27°27'20.1"	89°39'06.1"	2310	5	8.6	368
W7	2013/11/22	Simtokha river	27°26'18.6"	89°40'21.1"	2375	5	8.5	344
W8	2013/11/23	A house in Changjiji Residential Colony	27°27'14.5"	89°39'13.7"	2284	12	7.8	1
W9	2013/11/23	Changjiji Pump house	27°27'14.3"	89°39'13.7"	2230	12	7.7	None
W10	2013/11/23	Changjiji river tank	27°27'15.9"	89°39'23.2"	2312	12.5	7.7	2
W11	2013/11/23	Lungtenphu Primary School	27°27'27.8"	89°37'56.1"	2473	6	8.0	2
W12	2013/11/23	Changrigphel Lower Secondary School	27°26'48.3"	89°39'45.0"	2307	10	7.9	2
W13	2013/11/23	Changzamtok Lower Secondary School	27°27'42.2"	89°38'25.3"	2346	9	7.6	2



Technical Appendix Figure 1. Age distribution of norovirus (NoV) GII and GII.21 among children in Thimphu, Bhutan. Blue and red columns represent the number of patients with NoV GII and NoV GII.21, respectively among the indicated age categories.



Technical Appendix Figure 2. Phylogenetic tree of the capsid gene at the C region of noroviruses constructed by using nucleotide sequence by neighbor-joining method. For better view of the tree highly related strains of the following genotypes have been collapsed: GII.2, GII.6, and GII.3 are from Bhutan, and GII.21 is from other countries. Numbers adjacent to the nodes represent the bootstrap values. Values <50% are not shown. Each strain is identified by the source of detection followed by strain number, country of origin, and year of detection. Bhutan strains from humans and water sample are indicated by blue and red filled square, respectively. The DDBJ/EMBL/GenBank accession numbers for Bhutan strains are as follows: LC009578–LC009630. Scale bar indicates nucleic acid substitutions per site.