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Tandem Repeat Insertion in African Swine Fever Virus, Russia, 2012

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To the Editor: The recent introduction of African swine fever virus (ASFV) into the European Union (http://www.oie.int/animal-health-in-the-world/the-world-animal-health-information-system/data-after-2004-wahid/) has caused serious concern in pig industries in Europe and their trade partners. African swine fever is one of the most feared infections that can affect pig industries because no vaccine is available and the socioeconomic effect of an outbreak would be serious (*1*). Therefore, early detection and coordinated countermeasures are urgently needed. For these countermeasures, information on disease dynamics and evolution is mandatory. In this respect, molecular epidemiology can be used to trace virus spread and transmission pattern.

Because it is a DNA virus, ASFV evolves rather slowly, and the use of routine genome fragments (variable region of the B646L gene and parts of the E183L gene) for partial sequencing has so far shown 100% identity among strains found in Russia (*2*) and the neighboring countries (*3*). Thus, the resolution power of these approaches is too low for in-detail analyses, which depend on information regarding larger genome fragments or whole genomes.

In 2014, an insertion of a tandem repeat sequence (TRS) in the intergenic region between the I73R and the I329L protein genes was found in ASFV strains from Poland and Lithuania (*3*). This TRS insertion was also found in ASFV strains from Ukraine in July 2012 and from Belarus in June 2013, but not in strains from Russia, Georgia, or Azerbaijan. Gallardo et al. (*3*) concluded that ASFV strains in Lithuania and Poland most likely originated from Belarus. However, these authors indicated that for a full understanding of evolution and spread, additional sequence analyses would be needed, especially from regions of Russia bordering Belarus and Ukraine. We report information for 3 additional sequences from ASFV strains from Russia that were analyzed for the previously-mentioned TRS insertion on the basis of full-genome sequences.

These ASFV strains originated from domestic pigs from the Tulskaya oblast (Tula06/2012), the Tverskaya oblast Kashinskiy district (Kashinskiy 09/2012), and the Tverskaya oblast (Tver06/2012) in 2012. Genome sequences were obtained by using a primer-walking method that was adapted from Portugal et al. (*4*). Resulting PCR products were subjected to next-generation sequencing by using the MiSeq platform (Illumina, San Diego, CA, USA). Raw sequence data were analyzed and assembled by using Genome Sequencer software version 2.6 (Roche, Mannheim, Germany). Additional sequences of the intergenic region of 17 virus isolates from domestic pigs and wild boar from Russia were obtained by using conventional PCR, and amplicons were directly sequenced by using a 3130x1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommendations.

Chromatograms were manually edited and assembled by using CAP3 (http://pbil.univ-lyon1.fr/cap3.php). All nucleotide sequences of ASFV isolates obtained in this study were deposited in GenBank under accession nos. KP137625–KP137644. In the alignment, other published sequences available in GenBank from Poland, Lithuania, Belarus, Ukraine, Armenia, Azerbaijan, Russia, and Georgia were included (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/4/14-1792-Techapp1.pdf). Sequence alignment was performed by using the ClustalW algorithm (http://www.clustal.org) as implemented in Geneious version 7.1.7 (Biomatters Ltd., Auckland, New Zealand).

[1]These authors contributed equally to this article.

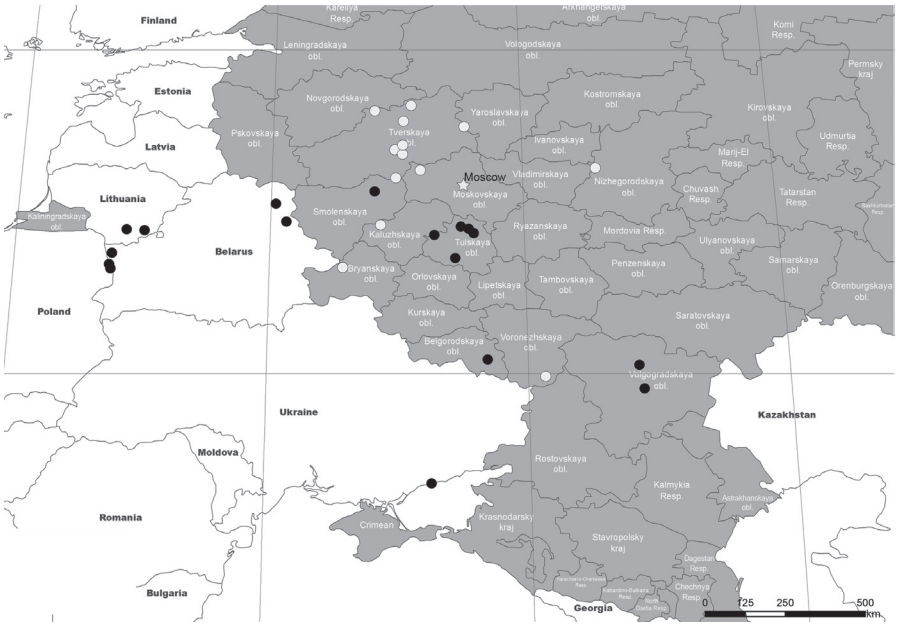

Figure. Locations where isolates of African swine fever virus were obtained in Russia during or after 2011. Black circles indicate isolates with tandem repeat insertions, and white circles indicate isolates without tandem repeat insertions. obl., oblast; Resp., respublika.

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 Tulskaya 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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Tandem Repeat Insertion in African Swine Fever Virus, Russia, 2012

Technical Appendix. Partial nucleotide alignment of African swine fever virus intergenic regions between I73R and I329L, Russia

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                                          10        20        30        40        50        60        70        80        90       100
                                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
1.Georgia_2007/1_FR682468         AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
2.Abk07_KJ620029                  AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
3.Arm07_KJ620028                  AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
4.Che07_KJ620030                  AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
5.Az08D_KJ620035                  AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
6.Az08B_KJ620036                  AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
7.Ing08_KJ620031                  AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
8.Oren08_KJ620032                 AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
9.NO08/Av_KJ620033                AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
10.NO08/Ap_KJ620034               AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
11.Dagestan09_KJ620048            AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
12.StPet09_KJ620050               AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
13.Kalmykia09_KJ620049            AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
14.Rostov09_KJ620051              AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
15.Tver0511/Torjo_KJ620038        AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
16.Tver0312/Torjo_KJ620041        AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
17.Tver0312/Novo_KJ620042         AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
18.Tver0712/Les_KJ620040          AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
19.Tver0812/Bolo_KJ620039         AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
20.Tver06/2012_KP137626           AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
21.Kashinskiy09/2012_KP137627     AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
22.Tula06/2012_KP137625           AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
23.Ukr12/Zapo_KJ620037            AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
24.Tula_06/13_KP137641            AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
25.Smolensk_06/13_KP137632        AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
26.Moscow_07/13_KP137629          AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
27.Voroneg_07/13_KP137635         AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
28.N.Novgorod_07/13_KP137628      AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
29.Tula_08/13_KP137633            AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
30.Smolensk_09/13_KP137634        AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
31.Bel13/Grodno_KJ620043          AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
32.Tula_01/14_KP137644            AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
33.Kaluga_01/14_KP137639          AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
34.Volgograd_01/14_KP137637       AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
35.Tula_02/14_KP137640            AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
36.Volgograd_02/14_KP137642       AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
37.Bryansk_02/14_KP137638         AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
38.Bryansk_05/14_KP137630         AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
39.Smolensk_05/14_KP137631        AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATA----------GGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
40.Belgorod_06/14_KP137636        AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
41.Kaluga_08/14_KP137643          AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
42.Lt14/1490_KJ620044             AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
43.Lt14/1482_KJ620045             AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
44.Pol14/Krus_KJ620047            AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
45.Pol14/Sz_KJ620046              AATAACACAGTTAAGCAATAAATAACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATACTAATT
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Technical Appendix Figure. Partial nucleotide alignment of African swine fever virus intergenic regions between I73R and I329L, Russia. -, regions without insertions. Blue indicates isolates without tandem repeat insertions, and red indicates isolates with tandem repeat insertions. Gray shading indicates isolates sequenced in this study. GenBank accession numbers are shown. Sequences in the alignment were from isolates from Poland (Pol14/Sz, Pol14/Krus), Lithuania (Lt14/1490, Lt14/1482), Belarus (Bel13/Grodno), Ukraine (Ukr12/Zap), Armenia (Arm07), Azerbaijan (Az08D, Az08B), Georgia (Georgia_2007/1, Abk07) and Russia (Che07, Ing08, Oren08, NO08/Av, NO08/Ap, Dagestan09, StPet09, Kalmykia09, Rostov09; Tver0511/Torjo, Tver0312/Novo, Tver0312/Torjo, Tver0712/Les, Tver0812/Bolo, Tver06/2012, Kashinskiy09/2012, Tula06/2012, Tula_06/13, Smolensk_06/13, Moscow_07/13, Voroneg_07/13, N.Novgorod_07/13, Tula_08/13, Smolensk_09/13, Tula_01/14, Kaluga_01/14, Volgograd_01/14, Tula_02/14, Volgograd_02/14, Bryansk_02/14, Bryansk_05/14, Smolensk_05/14, Belgorod_06/14, Kaluga_08/14).