Published in final edited form as:
Arch Virol. 2013 January ; 158(1): 281–282. doi:10.1007/s00705-012-1456-y.

Porcine kobuvirus in wild boars (*Sus scrofa*)

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Abstract

Fecal samples (N=10) from 6- to 8-week-old wild boar piglets (*Sus scrofa*), collected from an animal park in Hungary in April 2011, were analyzed using viral metagenomics and complete genome sequencing. Kobuvirus (genus *Kobuvirus*, family *Picornaviridae*) was detected in all (100%) specimens, with the closest nucleotide (89%) and amino acid (94%) sequence identity of the strain wild boar/WB1-HUN/2011/HUN (JX177612) to the prototype porcine kobuvirus S-1-HUN (EU787450). This study suggests that genetically highly similar (practically the same geno-/serotype) porcine kobuvirus circulate in wild boars, the wildlife counterparts of domestic pigs. Wild boars could be an important host and reservoir for kobuvirus.

The genus *Kobuvirus,* within the family *Picornaviridae*, consists of small, non-enveloped viruses with single-stranded, positive-sense RNA genomes ranging from 8.2 to 8.4 kb in length. Kobuviruses include members of two officially recognized species, *Aichi virus* (in humans) [13] and *Bovine kobuvirus* (in cattle) [12], and a candidate species, "Porcine kobuvirus" (in swine) [8]. More recently, kobuviruses have been also detected in sheep [10], dogs [6], canyon mice [7] and goats [5]. The diversity, distribution, pathogenicity, and host species spectrum of kobuviruses is still limited [9]; however, the increasing number of potential hosts raises the possibility that there are additional animals acting as hosts for kobuvirus replication.

In this study, using metagenomic analysis, we report the detection of a kobuvirus in fecal samples obtained from wild boars that is genetically very similar to porcine kobuvirus.

Fecal samples (N=10) from 6- to 8-week-old wild boars (*Sus scrofa*) were collected from an animal park located in southwestern Hungary in April 2011. None of the sampled boars showed any clinical symptoms at the time of sample collection. The boars were in captive breeding but had no contact with domestic pigs.

The fecal samples were subjected to viral metagenomics analysis as follows: Specimens diluted in 0.1 M phosphate-buffered saline (PBS) were passed through a 0.45-μm sterile

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Note: Nucleotide sequence data reported is available in the GenBank database under accession number JX177612.

The authors declare that they have no conflict of interest.

filter and centrifuged at 6,000 × *g* for 5 min. The pellet was mixed with a mixture of nucleases to enrich for particle-protected nucleic acids [4]. Nucleic acids were extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Viral RNA and DNA nucleic acid libraries were constructed by sequence-independent random RT-PCR amplification [14], and 454 pyrosequencing using 454 GS FLX technology was then performed as described previously [4, 14].

The pyrosequencing reads from the different samples were assembled *de novo*, and sequence contigs and singletons were compared to the GenBank nucleotide and protein databases using BLASTx. Specific primer pairs were designed based on the sequence contigs and singletons from the pyrosequencing reads to determine the complete nucleotide sequence of a selected kobuvirus using reverse transcription PCR (RT-PCR). The 5’ and the 3’ ends of the genome were determined using a 5’/3’ RACE PCR kit (Roche, Mannheim, Germany) as described previously [3]. PCR products were sequenced directly in both directions using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Warrington, UK) with specific primers and run on an automated sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems, Stafford, USA). The complete genome sequence of wild boar/WB1-HUN/2011/HUN was submitted to GenBank under accession number JX177612.

Porcine kobuvirus sequences were found in all (100%) of 10 fecal samples. The VP1 sequences of the wild boar strains showed 99% nucleotide (nt) and complete amino acid (aa) sequence identity to each other and 89% nt and 94% aa identity compared to the prototype (and the closest) porcine kobuvirus sequence S-1-HUN (EU787450) in the GenBank database.

One of the kobuvirus strains (WB1-HUN/2011/HUN) was selected for complete genome characterization. From this strain, 752 kobuviral sequence contigs and singletons with E-value $<10^{-10}$ using BLASTx were found (data not shown), covering 85% of the kobuviral genome. The complete genome length of wild boar/WB1-HUN/2011/HUN is 8210 nt excluding the poly(A) tail. A long ORF of 7467 nt, which encodes a 2488-aa-long potential viral polyprotein precursor, was flanked by a 576-nt-long 5’UTR and a 167-nt-long 3’UTR. The L, P1, P2 and P3 regions were 585 nt (195 aa), 2529 nt (843 aa), 1998 nt (666 aa) and 2355 nt (784 aa) long, respectively – the same lengths as those of porcine kobuvirus S-1-HUN. The amino acid identity between WB1-HUN/2011/HUN and S-1-HUN in the P1, P2 and P3 regions was 92%, 95% and 96%, respectively.

In this study, we report the detection of porcine kobuvirus in wild boars, the wildlife counterparts of domestic pigs. This study suggests that genetically highly similar (practically the same geno-/serotype) porcine kobuvirus circulate in domestic and wild boar piglets. In addition, porcine kobuvirus infection is probably as frequent in wild boars as it is in domestic pigs [9]. Interestingly, in addition to porcine kobuviruses, three other novel enteric RNA viruses, porcine teschovirus [2], porcine astrovirus [11] and porcine enterovirus [1], were detected – also at high frequency: 7 (70%), 5 (50%) and 5 (50%), respectively – in the 10 wild boar fecal samples collected, using metagenomic analysis. The observed co-infection with different RNA viruses indicates that wild boars could be an important host and reservoir for picorna- and astroviruses.

Acknowledgments

This work was supported by a grant from the Hungarian Scientific Research Fund (OTKA, K83013). G.R. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences; B.K. was supported by a fellowship from the Rosztoczy Foundation; and E.D., by NHLBI grant E01HL083254.

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