The characterization of complete genome of MERS-related coronavirus from bat (Pipistrellus nathusii) from Moscow region, prediction and analysis their Spike glycoprotein with DPP4 receptors of the different mammalian species

Speranskaya A.S.1,2*, Samoilov A.E.1, Korneenko E.V.1, Khabudaev K.V.1, Artyushin I.V.2, Yusefovich A.P.2, Dolgova A.S.3, Dedkov V.G.3

1 Research Institute for Systems Biology and Medicine of the on Consumers’ Rights Protection and Human Well-Being Surveillance, Moscow, Russia
2 Lomonosov Moscow State University, Biological Department, Moscow, Russia
3 Saint-Petersburg Pasteur Institute of the Federal Service on Consumers’ Rights Protection and Human Well-Being Surveillance, St. Petersburg, Russia

* hanna.s.939@gmail.com

Key words: Bat-CoV, MERS-related viruses, Pipistrellus nathusii, bats, hedgehogs, humans, camels, DPP4, Spike

Motivation and Aim: The majority of infectious diseases that were discovered during the last few decades are actually zoonosis. The ongoing pandemic disease COVID-19 is an infection caused by the severe acute respiratory syndrome coronavirus SARS-CoV-2. The animal reservoir of SARS-CoV-2 is unknown, but actively discussed as originating from bats, due to reports of SARS-CoV-2-related viruses in Asian Rhinolophus [1]. Previous severe human outbreak caused by coronaviruses was middle East respiratory syndrome (MERS). This disease has a high fatality rate of up to 35%. MERS was first identified in Saudi Arabia in 2012. Presently 27 countries have reported cases; the largest outbreaks were in Saudi Arabia, United Arab Emirates and the Republic of Korea. The origin of the MERS virus is not understood yet but supposedly it naturally circulated in bats and passed to humans through dromedary camels. Analysis of the virome of bats that are distributed in different geographical regions is an actual approach for identifying the new species of viruses that potentially cause human infection. Currently, a large number of MERS-related coronaviruses from bats of China, Asia, Near East, some countries of Europe and Africa are described. But on the territory of the Russian Federation the MERS-related coronaviruses of bats were not found earlier. We have been screening bat colonies in the Central Region of Russian Federation during the last few years. Some time ago we found the novel MERS-related betacoronavirus in the wild bat Pipistrellus nathusii, which was caught near Moscow in 2015. We sequenced and assembled the complete genome of this novel virus. The aim of this work – to better understand the viral adaptability to host as well as various other potential hosts and to estimate the possibility of viral transmission to mammalian species which could contact with humans using computer analysis methods like phylogenetic and phylogeographic analysis, comparative nucleic acid and protein motif analysis and protein-protein docking.

Methods and Algorithms: The whole genome sequencing of novel MERS-related Betacoronavirus from Pipistrellus nathusii was performed using Illumina sequencing. The complete genome was assembled. We described its genome organization features,
relationship with known coronaviruses, estimated the possibility of viral-host coevolution. Genome annotation was performed by Geneious 7.1.9 and manually edited. For phylogenetic analysis, the independent sampling of available GenBank records for complete genomes, S-, N- and RdRp-encoding sequences was performed, using “Merbecovirus” keywords as the primary filter. The alignments were constructed using MAFFT v7 and phylogenetic analyses were performed using online W-IQ-TREE (ModelFinder + tree reconstruction + ultrafast bootstrap of 1000 replicates). The three-dimensional structure of the S-protein of novel merbecovirus as well as DPP4 receptors for Myotis brandtii (EPQ03439.1), Pipistrellus kuhlii (KAF6353216.1), Erinaceus europaeus (XP_016043930.1), Felis catus (NP_001009838.1) and Mus musculus (NP_034204.1) was modeled on the SWISS-MODEL server. The RBD 360-610 a.a. of Spike-protein and all DPP4 sites of the studied organisms were used to calculate the docking using the HDOCK server and analyzed in PyMol.

Results: The novel virus was named MOW-BatCoV strain 15-22 and deposited in the GenBank database under accession numbers ON325306. The genome organization of MOW-BatCoV/15-22 virus was found identical to that of other known MERS-related CoV viruses and encompassing the open reading frames (ORFs) in the following order: ORF1ab–spike(S)-ORF3-ORF4a-ORF4b-ORF5-envelope(E)-membrane(M)-nucleocapsid(N)-ORF8b. The trees constructed using complete genomes, N- and RdRp-sequences show the three distinct phylogenetic clades: I - consists of viruses from hedgehogs, II - viruses from the bat only, III - viruses of the bat, humans and camels. The novel MOW-BatCoV/15-22 falls into Clade III and formed a distinct subclade closely related to human/camel’s viruses together with MERS-related coronaviruses of bats Hypsugo savii and Pipistrellus kuhlii (from Italy) and from Neoromicia capensis (from South Africa). Unexpectedly, the results of a phylogenetic analysis of the Spike-genes showed differing results, namely: the closest similarity of two viruses from bats - MOW-BatCoV 15-22 and Neoromicia/5038 - with coronaviruses from Erinaceus (hedgehogs) was determined. Using the computer molecular docking we analyzed the binding of Spike glycoprotein vs DPP4 receptors of different mammalian. The highest binding was predicted in the interaction of MOW-BatCoV Spike-protein and DPP4 of the bat M. brandtii (docking score -320.15). Among the other organisms, the highest binding was predicted for the hedgehog, E. europaeus (docking score -294.51). The docking results were consistent with the results of the phylogenetic analysis performed. The recent investigation showed MERS-related coronaviruses in hedgehogs from some countries of Europe as well as in China, suggesting that hedgehogs may represent a wild reservoir of novel betacoronovirus, subgenus Merbecovirus in wild hedgehogs [2]. Hedgehogs are animals with cute faces and rising popularity as pets. Besides, humans contact them in the wild. We suggest that hedgehogs may be potential intermediate hosts for viruses between bats and humans.

Acknowledgements: The zoology, molecular virology and bioinformatician works were supported by Grant RFBR No. 20-04-60561. The molecular docking works were supported by the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing state task No. 122030900051-9.

References