Type: Poster Presentation

Final Abstract Number: 43.236
Session: Poster Session III
Date: Saturday, March 5, 2016
Time: 12:45-14:15
Room: Hall 3 (Posters & Exhibition)

Phylogenetic analysis of the complete genome of the APMV-13 isolate from Ukraine

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Background: Avian paramyxoviruses (APMVs) belong to the genus *Avulavirus* of the family *Paramyxoviridae* and have been shown to infect a wide variety of poultry and wild bird species. Up to date, the International Committee on Taxonomy of viruses have officially approved 9 serotypes of APMV (APMV 1 – 9), but recently four new serotypes have been described (APMV 10 – 13). A member of the putative new serotype APMV 13 was isolated from white-fronted goose on the territory of Askania-Nova National Park (Ukraine) in 2011.

Methods & Materials: The virus was propagated in specific-pathogen-free embryonated chicken eggs and preliminary analyses of the isolate were performed by Sanger sequencing. Based on the obtained results, primers were designed: APMV13-3000 CTGTCGCAGTAATGGAAGGGAA/BIOTIN, APMV13-6000 GGGAAGC-CCTTCTCTTGATTGAT/BIOTIN and APMV13-12000 GCTGGACT-GCGCCTATCAAAT/BIOTIN/ and isolate was further processed by next-generation sequencing (NGS). The phylogenetic analysis was performed with the software MEGA6.06 and the evolutionary history was inferred by using 52 full genome sequencing data of APMVs and the Neighbor joining methods, bootstrap 1000 (Tamura, Stecher, Peterson, Filipski, and Kumar 2013).

Results: The complete genome contains six transcriptional units (3'-NP-P-M-F-HN-L-5') of 1482, 1194, 1101, 1653, 1740 and 6639 nucleotides in length, respectively. Amino acid based phylogenetic analysis of coding regions revealed closest relationship to APMV 12, however low amino acid identity (52% to 75%) was observed. The fusion protein gene of the virus has 98% nucleotide identity to APMV/goose/Shimane/67/2000, isolated from a fecal sample collected from a goose and reported previously as APMV 13. Phylogenetic evaluation of coding regions revealed that predicted amino acid sequences of all other proteins were more closely related to APMV-12 (Np has 75.1% sequence identity, P-52.1%, M-73.1%, F-69.5%, HN-63.7%, L-66.0%) than to the rest of the serotypes.

Conclusion: Our data highlights the importance of continuous monitoring of wild birds in the Azov-Black Sea region and to further identify possible introductions of avian viruses from other geographic regions.

This project was partially funded by Project Agreement P-568 funded by the Department of State, and by the Defense Threat Reduction Agency USDA/ARS #685/FRCALL 12-6-2-0005.

http://dx.doi.org/10.1016/j.ijid.2016.02.972

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Characterisation of chronic hepatitis B virus carriers with viral load and correlation with other viral markers

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Background: According to WHO, India has around 40 million chronic HBV carriers. This study was carried out with objectives; profiling of viral markers in HBV carriers with viral load, correlation of ALT levels, HBe Ag status with their viral load, to determine the mutation and response to antiviral therapy in treated and treatment naïve individuals.

Methods & Materials: DNA was extracted from plasma by Qiagen DNA blood mini kit (Qiagen, Germany). HBV viral load was estimated by Artus HBV real time PCR kit (Qiagen, Germany) in ABI HT fast real time PCR platform (Applied Biosystems, USA). Randomly 27 high viral load samples were chosen and polymerase gene was amplified using specific primers by Platinum Taq DNA polymerase kit (Invitrogen, USA) and sanger sequencing (Big Dye terminator kit, ABI, USA) was performed to identify the drug resistance mutations and genotyping. Mutational analysis was done by HBV geno2pheno software.

Results: Among 1129 samples tested for viral loads, 26% (n=295) had high viral load (Median HBV viral load is 7×10^5 IU/ml, range: 2×10^3 IU/ml to 4×10^7 IU/ml), of which 113 samples had viral load between 2000 IU/ml -20,000 IU/ml and 182 had more than 20,000 IU/ml. 31% had detectable viral load below 2000 IU/ml.. HBe Ag status were checked for these patients, HBe Ag status were known for 545 patients, 26% (n=141) were found positive for HBe Ag, 13.7% had high viral load with HBe Ag negative. HBe Ag positivity was positively correlated with the high viral load with $p<0.001$ (STATA II software). Elevated ALT levels were seen in patients with high viral load and the correlation was significant with $p<0.001$. Among 27 individuals, mutation rtM204V along with rtL180M was seen in one treated individual and a compensatory mutation was observed in other treatment naive individual.

Conclusion: Periodic monitoring of these patients for factors like viral load, HBe Ag status and ALT level will enable the clinician to initiate appropriate therapy at the right time to obtain sustained virological response.

http://dx.doi.org/10.1016/j.ijid.2016.02.973