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Characterization and Phylogenetic Analysis of Hemagglutinin & Neuraminidase Genes of Avian Influenza Virus Subtype H9n2 in Pakistan

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Despite extensive vaccination, H9 Avian influenza outbreaks has caused great economic losses to poultry industry resulting in decrease egg production, high morbidity and mortality. The ability to cross species barrier makes it a potent threat. Continuous mutations in the HA gene transforms AIV subtype H9N2 into more pathogenic virus that may have pandemic potential and can cross species barrier. Thus, it is essential to continuously monitor antigenic variants of H9 virus. HA gene plays vital role in viral attachment, release of genetic material and pathogenicity. In present study, a sum of four H9 virus samples were isolated, serological and molecular confirmation was done. 500 samples were collected and properly labelled. They were then processed for egg inoculation in embryonated eggs. Virus was grown in embryonated eggs and harvested fluid is then preceded for confirmatory testing. Haemagglutination and Haemagglutination Inhibition testing was done. RNA was extracted by Kit method and cDNA was synthesized. Reverse Transcriptase (RT-PCR) was performed using specific primer sets and then the amplicon were run on agarose gel. PCR product was sent for sequencing and Phylogenetic tree was constructed. The present study enabled us to characterize and construct Phylogenetic tree of HA gene of currently prevailing H9N2 Avian Influenza isolates in Pakistan belongs to G1-like sub lineage. Amino acid analysis revealed substitution of polybasic amino acid residues that my transform H9N2 into more pathogenic.