AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

AUTHOR REPLY

Reply to "Complexities of Estimating Evolutionary Rates in Viruses"

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We appreciate the valuable feedback offered by Dr. Holmes (1) and would like to follow up on some of the important points. The goal of our study was to evaluate the feasibility of identifying areas in which to focus surveillance efforts to better detect the emergence of new influenza strains and subtypes, using the currently available data, both for potential targeting of future surveillance and for identifying data gaps necessary to make such informed decisions. We concur that the estimation of virus evolution rates can be challenging, especially for a virus such as influenza A, which can infect numerous different, mobile animal groups. We must keep in mind that even under optimal circumstances (i.e., a single host group, large number of sequences, long time interval, etc.), nucleotide substitution rate calculations are only estimates. Additionally, analyses such as these, which admittedly have inherent limitations, are extremely useful for demonstrating data gaps and helping to push for the advancement of the science necessary to improve global surveillance.

In conducting our analysis, we were aware of, and acknowledged, the limitations inherent in estimating substitution rates, especially at small geographical scales and with limited numbers of sequences (2). In fact, for the majority of subtypes analyzed, a lack of available sequences from many individual countries severely limited the utility of geographic clustering analysis. That is why in addition to performing a country-level analysis, we calculated nucleotide substitution rates at a regional scale. For all substitution rates estimated, we provided a measure of statistical uncertainty (95% highest probability density [HPD] values). At the country level, particularly for those countries with limited numbers of sequences (i.e., Mongolia and Pakistan for H5N1), we noted that there was a greater interval of statistical uncertainty than in countries with larger numbers of sequences (i.e., China and Vietnam). The considerable discrepancies in available sequence data from different countries led us to strongly recommended more-extensive testing for and reporting of all potential subtypes worldwide and especially in underrepresented areas.

The nucleotide substitution rates calculated in our study ranged from a minimum of 1.43×10^{-3} (H3N8) to a maximum of 11.62×10^{-3} (H7N7). Overall, the substitution rates that we calculated were quite similar to and consistent with those calculated by others using similar analytical methods. For example, Chen and Holmes (3) estimated the H6N2 hemagglutinin (HA) subtype at a global scale to have a substitution rate of 4.63×10^{-3}, while our analysis provided estimated rates of 2.04×10^{-3} for the United States and 5.27×10^{-3} for China. For avian H3N8 strains, Chen and Holmes calculated a global rate of 2.06×10^{-3}, while our estimates ranged from 1.68×10^{-3} to 3.69×10^{-3}. For the H7 subtype, Lebarbenchon and Stallknecht (4) estimated rates of 11.21×10^{-3} from Sweden and 11.74×10^{-3} for North America. The rates that we estimated for H7N7 strains were 10.49×10^{-3} for Sweden and 11.62×10^{-3} for North America. Finally, for H5N1 strains, G. Cattoli et al. (5) estimated substitution rates of 5.36×10^{-3}, 5.20×10^{-3}, 4.04×10^{-3}, and 2.52×10^{-3} for isolates from Egypt, Nigeria, Turkey, and Thailand, respectively. The rates that we calculated were 4.22×10^{-3} (Egypt), 5.48×10^{-3} (Nigeria), 3.03×10^{-3} (Turkey), and 2.32×10^{-3} (Thailand).

The nucleotide substitution rates calculated in our study were part of a larger effort to try to identify trends in the evolutionary dynamics of influenza A virus at various spatial scales. One of the conclusions of our analysis, that nucleotide substitution rates were higher for subtypes (H5N1, H5N2, and H6N1) in East Asia than in North America, was actually based on a regional analysis, as Dr. Holmes has recommended in his letter, which showed statistically significant differences (no overlaps in 95% HPDs) between these regions. Although the country-level analyses for these and other subtypes showed similar trends, a lack of sequences for many countries and subtypes resulted in greater statistical uncertainty, and we did not claim those results to be significant. We also agree that influenza virus diversity is not affected by geo-political boundaries, and thus regional analyses, as we presented, are more informative for targeting surveillance efforts.

In conclusion, the methods that we used to estimate substitution rates were well established, we provided statistical uncertainty (95% HPD) values, and the rates calculated were comparable to those presented in other studies. By attempting to make predictions about geographic locations key for influenza A virus evolution and emergence, we identified and concluded that current gaps in influenza A virus surveillance and reporting make such predictions challenging at this time.

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Citation Rejmanek D, Hosseini PR, Mazet JAK, Daszak P, Goldstein T. 2016. Reply to "Complexities of estimating evolutionary rates in viruses." J Virol 90:2156. doi:10.1128/JVI.02746-15.

Editor: D. S. Lyles

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This is a response to a letter by Holmes (doi:10.1128/JVI.02570-15).