Genome-Wide Evolutionary Analyses of G2P[4] Rotaviruses Circulating Before and After Rotavirus Vaccine Introduction in Ghana, Malawi, and South Africa

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Background: G2P[4] is an important rotavirus genotype that has fluctuated in prevalence in African countries over time. There is a paucity of sequencing of African G2P[4] strains. To address this, the African Enteric Viruses Genome Initiative (AEVGI) has conducted whole-genome characterization of rotavirus strains from South Africa, Ghana, and Malawi countries; pre- and post-vaccine introduction to gain insights into the genetic diversity and evolution of this genotype following Rotarix introduction.

Methods: Full-length whole genomes were resolved for G2P[4] strains that circulated in children <5 years before and after vaccine introduction in Ghana (2008-2012 n=30, 2013-2019 n=61), Malawi (2008-2014 n=70, 2015-2018 n=41) and South Africa (2003-2009 n=46, 2010-2017 n=57). Time-measured evolutionary history and relative genetic diversity were resolved using Bayesian phylogenetic inference implemented in BEAST.

Results: The times to the most recent common ancestor (tMRCAs) estimated for each of the 11 genes of Malawian G2P[4] strains ranged between 2007.3 and 2007.7 and rates of evolution ranged between $1.10x10^{3}$ to $3.83x10^{3}$ nucleotide substitutions/site/year. The tMRCAs of the South African rotaviruses ranged between 1998.7 and 2001.9 and the rates of evolution ranged between $1.02x10^{3}$ to $2.41x10^{3}$ nucleotide substitutions/site/year. The VP1, VP3 and NSP4 genes of Malawian and South African strains exhibited no temporal signal due to the co-circulation of highly divergent variants. Frequent virus migration was observed between Malawi and South Africa in contrast to distinct lineages largely confined to Ghana. The tMRCAs of the Ghanaian rotaviruses ranged between 2004.3 and 2007.2 and rates of evolution ranged between $1.09x10^{3}$ to $3.35x10^{3}$ nucleotide substitutions/site/year. The population size estimates for all gene datasets revealed a relatively stable profile with minor fluctuations in diversity occurring before and after vaccine introduction.

Conclusion: Current data suggests that vaccine introduction has not drastically altered the pattern of evolution of G2P[4] strains in Malawi, Ghana, and South Africa. Regional variants continue to evolve supplemented by the introduction of globally circulating variants. Continued surveillance of G2P[4] strains in the region is required to further elucidate the complex patterns

of transmission between African countries where endemic variants circulate in addition to globally circulating variants and ascertain any long-term effects of vaccine introduction.