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Spatiotemporal dynamics of influenza A(H1N1)pdm09 in Brazil during the pandemic and post-pandemic periods

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ABSTRACT

Influenza A(H1N1)pdm09 was responsible for the first global flu pandemic in 21st century affecting all the world. In Brazil, A(H1N1)pdm09 is still circulating as a seasonal virus, causing deaths every year. Nevertheless, the viral diffusion process that yearly seeds new influenza strains in the country was not investigated yet. The aim of the current study was to describe the phylodynamics and phylogeography of influenza A(H1N1)pdm09 in Brazil between 2009 and 2014. Neuraminidase sequences from Brazil and other regions of the World were retrieved and analyzed. Bayesian phylogeographic and phylodynamic model approaches were used to reconstruct the spatiotemporal and demographic history of influenza A(H1N1)pdm09 in Brazil (divided in subtropical and tropical regions) and related countries. Our analyses reveal that new influenza A(H1N1)pdm09 lineages are seeded in Brazil in almost each year and the main sources of viral diversity are North America, Europe and East Asia. The phylogeographic asymmetric model also revealed that Brazil, mainly the subtropical region, seeds viral lineages into other countries. Coalescent analysis of the compiled dataset reconstructed the peak of viral transmissions in the winter months of Southern hemisphere. The results presented in this study can be informative to public health, guide intervention strategies and in the understanding of flu virus migration, which helps to predict antigenic drift and consequently the developing of new vaccines.

1. Introduction

Influenza is an acute viral infection that affects the respiratory system and has a global high transmission power (World Health Organization, 2009). In 2009, influenza A(H1N1)pdm09 was responsible for the first human global flu pandemic in the 21st century (Centers for Disease Control and Prevention, 2009). This virus was originated from at least four reassortment events among classical swine H1N1 and two or more North American avian and human H3N2 lineages (Smith et al., 2009). It emerged in North America in April and shortly thereafter more than 375,000 cases had been confirmed in 208 countries (World Health Organization, 2009). In Northern hemisphere, influenza A(H1N1)pdm09 initially disseminated outside the winter season in the first year of pandemic. On oppose, a full A(H1N1pdm)09 epidemic was observed in the most temperate regions of the South continents in the same year, replacing the seasonal strains in many countries (World Health Organization, 2009).

Brazil has the biggest population in the South hemisphere. Although most of its territory lies in the tropics, more than 60% of the population lives in the subtropical area with mild to cold winters (Brazilian Institute of Geography and Statistics, 2016). The first Brazilian case of A(H1N1)pdm09 occurred in May and sustained transmission was reported in July 2009, with the highest incidence rate in the South and Southeast regions (Secretary of Health Surveillance, 2009). Since then, influenza A(H1N1)pdm09 is still circulating as a seasonal virus together with influenza A(H3N2) and B virus lineages worldwide (World Health Organization, 2016). The same scenario is observed in Brazil and cases of infection by A(H1N1)pdm09 have been reported every year with an increasing frequency in the winter period (Secretary of Health Surveillance, 2016).

Phylogenetic analysis revealed that at least seven A(H1N1)pdm09 distinct clades arose in the initial global spread (Nelson et al., 2009). In Brazil, clades 5–7 circulated in the pandemic period (Goñi et al., 2011; Oliveira et al., 2014; Sant'Anna et al., 2014), reflecting multiple introductions and origins. Moreover, other six phylogenetic groups were found in the subsequent epidemic seasons from 2011 to 2014 (Resende

Abbreviations: NA, neuraminidase; HA, hemagglutinin; ML, maximum likelihood; MLE, marginal likelihood estimation; HPD, highest posterior density; MCMC, Monte Carlo Markov Chains; ESS, effective sample size; BSSVS, Bayesian stochastic search variable selection; BF, Bayes Factor; pp, posterior probability

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et al., 2015), suggesting a complex dynamic of new lineages seeded into the country at each influenza season.

Spatiotemporal dynamics of the influenza A(H3N2) are well characterized. Studies have demonstrated that new lineages are yearly seeded from tropical East and South-East Asia countries into temperate regions and that air passengers flows can predict global influenza migration (Russell et al., 2008; Rambaut et al., 2008; Lemey et al., 2014). However, few studies have investigated the global diffusion pattern of influenza A(H1N1), including the A(H1N1)pdm09 strain (Su et al., 2015).

In the current study we analyzed the phylodynamics of influenza A(H1N1)pdm09 in Brazil between 2009 and 2014. We inferred the demographic history of each seasonal epidemic inside the country and reconstructed the spatiotemporal dynamics of new lineages being seeded into Brazil and between tropical and subtropical regions within the country. Our results reveal that the main sources of A(H1N1)pdm09 diversity in Brazil are North America, Europe and East Asia. The current study also highlights the role of Brazil in seeding A(H1N1)pdm09 lineages into other countries, mainly in the Americas.

2. Materials and methods

2.1. Sequence dataset compilation

All Neuraminidase (NA) and Hemagglutinin (HA) sequences from A(H1N1)pdm09 isolated in Brazil were downloaded from Influenza Research Database (www.fludb.org). To compile a dataset representative from the pandemic and post-pandemic period, NA and HA sequence datasets were analyzed by sampling distribution over time. While HA dataset was mostly composed by samples from 2009, NA sequences were well distributed over 2009 and 2014. Therefore only sequences from NA were analyzed in the current study.

Sequences from other countries were selected by using BLAST+ (Camacho et al., 2009), where all international A(H1N1)pdm09 NA sequences available in public databanks were searched against the Brazilian dataset. The best 100 hits per query were added in the dataset for further analyses. Sequences were aligned using Mafft (Katoh and Standley, 2013) and visually inspected in AliView (Larsson, 2014) in order to trim out the alignment and delete short sequences. RAxML (Stamatakis, 2014) was used to infer an exploratory maximum likelihood (ML) tree and to identify and remove identical sequences. Finally, ML trees were analyzed in Tempest software (Rambaut et al., 2016) and sequences outliers in a regression of root-to-tip divergence versus sampling time were removed. This procedure resulted in a dataset with 1430 A(H1N1)pdm09 NA sequences (186 from Brazil and 1240 from worldwide).

2.2. Bayesian phylogenetic analysis

In order to diminish computational burden of the Bayesian analyses, the dataset was randomly down-sampled to around 500 sequences following two strategies: 1) Brazilian sequences were down-sampled proportionally by year of collection; 2) International sequences were down-sampled aiming to reduce the heterogeneity of samples by location. Supplementary Dataset 1 contains details about all sequences used in the Bayesian analyses. Time-scaled phylogenetic tree reconstruction was performed using BEAST/BEAGLE software (Drummond et al., 2012; Ayres et al., 2012) as available in the Cipres Science Gateway (https://www.phylo.org). Marginal likelihood estimation (MLE) (Baele et al., 2012; Baele et al., 2013) was applied to compare alternative site and clock models in a Bayesian framework. Trees were reconstructed using SRD06 substitution model (Shapiro et al., 2006) and the uncorrelated lognormal distribution relaxed molecular clock (Drummond et al., 2006), which outperformed alternative models. To allow for multiple changes in the viral effective population size across time, the non-parametric Bayesian skyride coalescent model was applied in the analyses (Minin et al., 2008). Moreover, demographic history of the influenza A(H1N1)pdm09 epidemic was estimated for the Brazilian NA dataset, using the same models as in the complete dataset analysis but testing the best coalescent model. Five Monte Carlo Markov Chains (MCMC) were run in parallel for 100 million states and combined in LogCombiner software (http://beast.bio.ed.ac.uk/LogCombiner) after excluding the burn-in. Tracer software (http://beast.bio.ed.ac.uk/Tracer) was used to diagnose MCMC, adjust initial burn-in and to summarize demographic reconstruction.

2.3. Phylogeographic analysis

A representative sample of 1000 trees was collected from the Bayesian phylogenetic analysis and used as an empirical tree distribution for estimating the virus migration process. Phylogeographic analysis was performed by applying a discrete asymmetric model of location transitioning with the Bayesian stochastic search variable selection (BSSVS) procedure (Lemey et al., 2009). Due to the high number of locations (N = 46), countries were grouped into 12 regions according to the continental localization (Supplementary Dataset 1). To account for the internal heterogeneity of influenza dynamics, Brazil was divided into tropical (BRA-trop) and subtropical (BRA-sub) regions and the respective sequences were assigned according the federal state where they were sampled, totalizing a model with 14 geographical locations. Phylogeographic analysis was complemented with Markov jump estimation of the number of location transitions throughout the evolutionary history (O'Brien et al., 2009). SPREAD software was used to identify the well-supported transition rates based on Bayes Factor (BF) support higher than three. RStudio (http://www.rstudio.org/) was used to summarize from BEAST log files the number of transitions (Markov Jump counts) between each of the geographical regions averaged over the posterior distribution of trees. For the highly supported transitions, the total number of jumps was plotted over time using frequency polygons.

3. Results

Bayesian phylogenetic analyses estimated that influenza A(H1N1)pdm09 was introduced into Brazil in February 2009 (October 2008–March 2009, 95% HPD), with the highest posterior probability (pp = 0.95) to North America as being the original location (Supplementary Fig. 1). Phylogeographic reconstruction of ancestral locations estimated North America, Europe and East Asia as the main sources of viral diversity in Brazil with some differences between tropical and subtropical regions regarding the dynamics of seeding lineages (Fig. 1). Subtropical Brazil (BRA-sub) was the destination of a high amount of transitions from North America in 2009. These transitions are mainly distributed around the winter months in Southern hemisphere, when the epidemic was well established in the world. Europe also contributed to seeding viral lineages into BRA-sub in 2009, but in a much smaller scale than North America. Tropical Brazil (BRA-trop), by its turn, received a small number of influenza A(H1N1)pdm09 lineages in 2009. These transitions were also centered on the middle of the year and the origins were North America, East Asia and Brazilian subtropical region.

In the post-pandemic period, North America and Europe had a similar role in introducing new lineages of influenza A(H1N1)pdm09 into BRA-sub. The seeding process was concentrated in the beginning of the year (the winter period in Northern hemisphere) rather than in the winter months of the Southern hemisphere as was observed in 2009 (Fig. 1). In 2011, Middle East region was also observed to contribute with some viral diversity to BRA-sub with transitions being supported by a slightly significant Bayes Factor score (BF = 3.5). From 2012 to 2014 few introductions of viral lineages into BRA-sub was reconstructed in our analysis and the origin was mainly North America. Despite some contribution of North America and East Asia, in 2012 the