Phylodynamics of Influenza A/H1N1pdm09 in India Reveals Circulation Patterns and Increased Selection for Clade 6b Residues and Other High Mortality Mutants

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ABSTRACT SUMMARY

- The severity of influenza A/H1N1pdm09 infection in India is unusually high, particularly in 2015 and 2017.
- We use phylogenetic and phylodynamic methods to uncover genetic explanations for this while also identifying patterns and drivers of transmission.
- We found increased selection at a number of hemagglutinin (HA) sites associated with increased morbidity and mortality relative to isolates circulating globally (Table 1).
- Residue changes at sites 84, 163, 185 and 256 are characteristic of clade 6b A/H1N1pdm09 strains.
- Increased selection at site 186 is rarely reported in the literature. We propose mutants at this site, such as A186T, may be novel determinants of A/H1N1pdm09 severity (Table 1).
- We show the state of Maharashtra is a critical for A/H1N1pdm09 spread throughout India indicating opportunities for epidemic control (Figure 2).
- Domestic airline travel is shown as a key factor driving spread among other climactic and ecological factors (Table not shown).
- High case under-ascertainment during mild flu years, e.g. 2014 supports the conclusion that a antigenically novel A/H1N1pdm09 virus emerged in the population in during the severe 2015 season. (Figure 2).
- Our results have important implications for future A/H1N1pdm09 surveillance and control within India, but also for epidemic and pandemic risk prediction around the world.

METHODS

We collected all full-length (>1,600) HA sequences sampled in India between 2009-2017 with collection date and location metadata publicly available or available upon request (n=625) from the Global Initiative for the Sharing All Influenza Data (GISAID). We removed 12 sequences across five State and Union Territories (S/UT) due to low sampling frequencies, leaving 613 sequences for analysis. For comparison we searched GISAID for all HA sequences sampled globally excluding duplicates identifying 21,209 sequences aggregated to one of ten regions. We generated two independent subsets (2 x n = 4,063) randomly sampling up to 50 sequences per region-year to reduce the impact of sampling bias and computational burden. Bayesian model testing fit a GTR+Γ4 substitution model with a relaxed molecular clock to both datasets. We generated ratios of non-synonymous to synonymous (d_N/d_S) mutations in BEAST v1.10. Sites under selection were validated using both two-rate fixed effects likelihood (FEL) and single-likelihood ancestor counting (SLAC) tests in HyPhy assuming a p-value threshold of 0.1. We used BEAST v1.10 to specify a discrete-trait phylogeographic model to estimate all possible transitions between the 14 Indian S/UT included in our dataset. A nonparametric Bayesian Skygrid tree prior with 50 intervals as default was used to reconstruct past population demographics. We used a generalised linear modelling (GLM) framework in Beast v1.10 to investigate the contribution of climactic, ecological, and demographic factors as potential predictors of transmission. Factors tested included state population density, average temperature, longitude, commercial passenger flux and pair-wise distance between locations.

Table 1. d_N/d_S ratios of codon sites identified in India under positive selection relative to d_N/d_S ratios of two distinct international samples.

Site (H3[#])	Ag	India Taxa (n = 613)		International Taxa (2 x n = 4,063)		Sig
		d_N/d_S	95% BCI	d_N/d_S	95% BCI	
84 (92)	*n*/a	8.14	(5.68–10.92)	3.02	(1.69–4.79)	*
163 (166)	Sa	3.83	(2.68–5.35)	3.58	(1.79–6.38)	
185 (188)	Sb	4.50	(3.18–6.21)	1.64	(0.76–2.94)	*
186 (189)	Sb	3.35	(2.26–4.51)	1.46	(0.93–1.97)	*
222 (225)	Ca	13.42	(9.43–18.42)	3.87	(2.30–5.85)	*
256 (259)	*n*/a	4.39	(3.12–6.09)	1.12	(0.74–1.58)	*

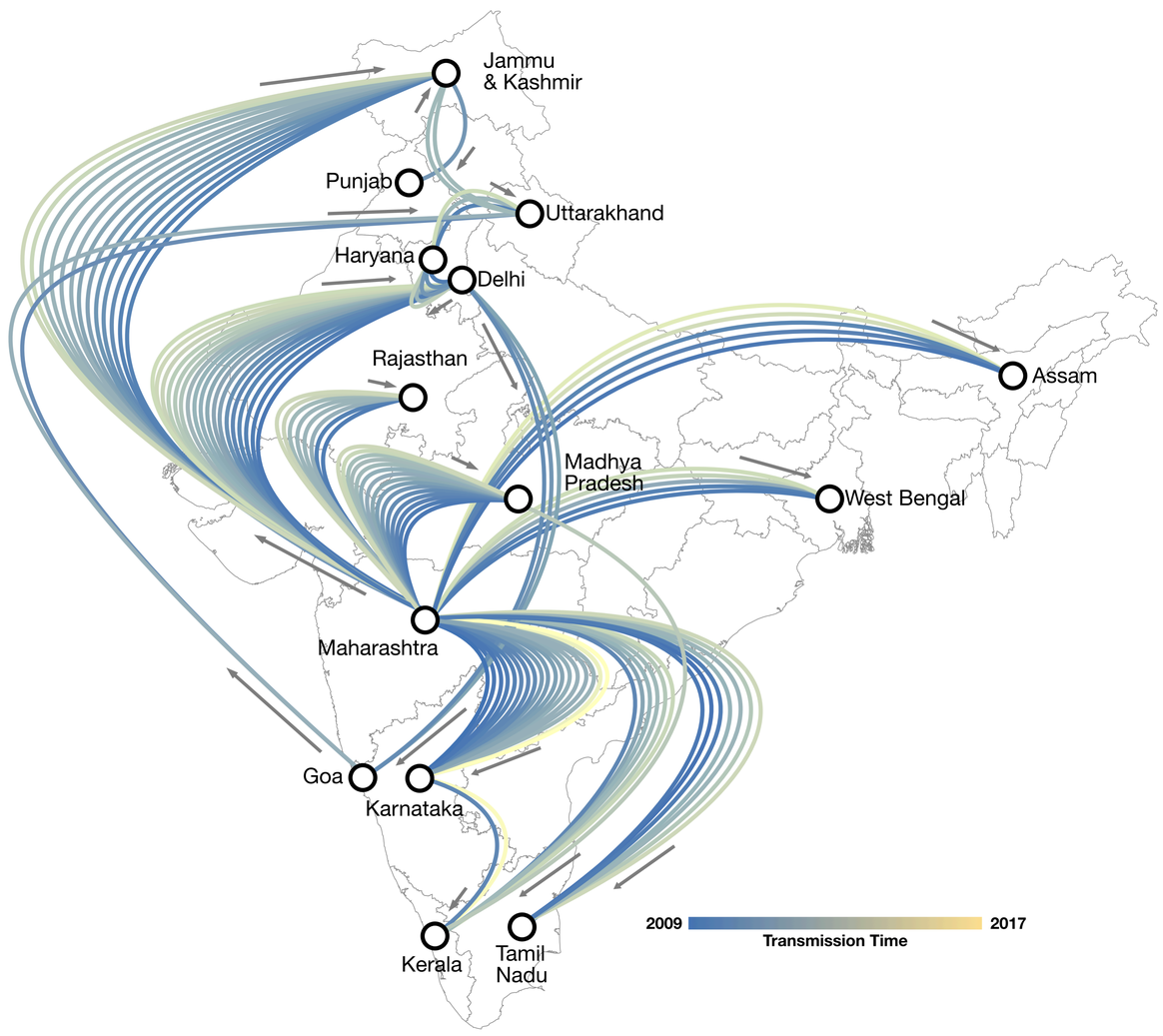

Figure1. Phylogeography of definitive A/H1N1pdm09 transmission between S/UT in India

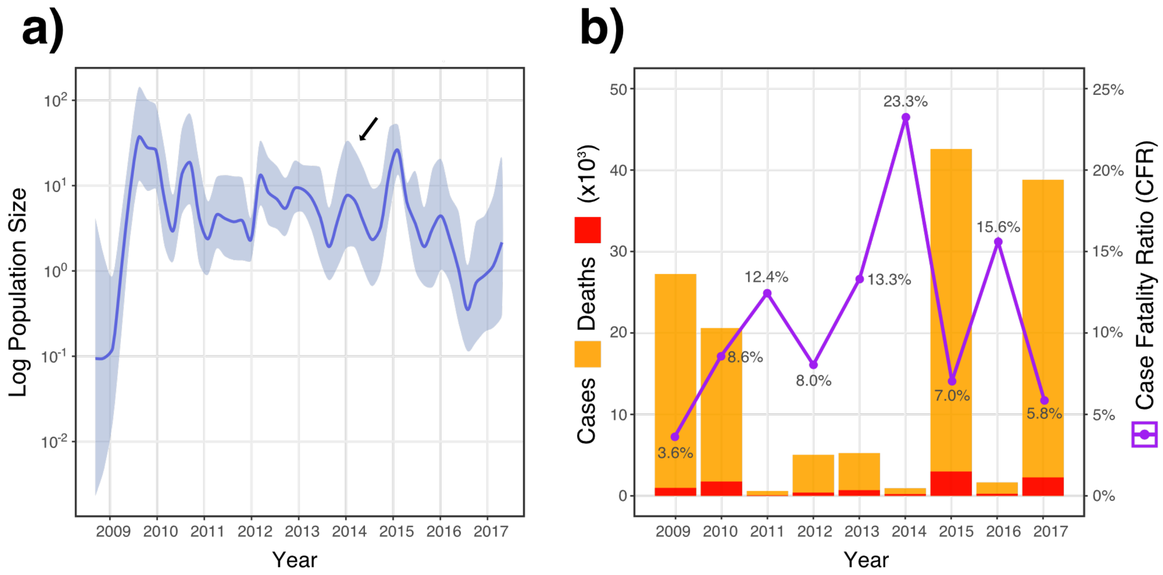

Figure 2. a) Effective viral population size (N_e) of A/H1N1pdm09 in India approximates yearly epidemic curves. **b)** Reported A/H1N1pdm09 cases, deaths and CFR (Right).

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