

## Supplementary Materials

# Genetic Characterization of Highly Pathogenic Avian Influenza A(H5N8) Virus in Pakistani Live Bird Markets Reveals Rapid Diversification of Clade 2.3.4.4b Viruses

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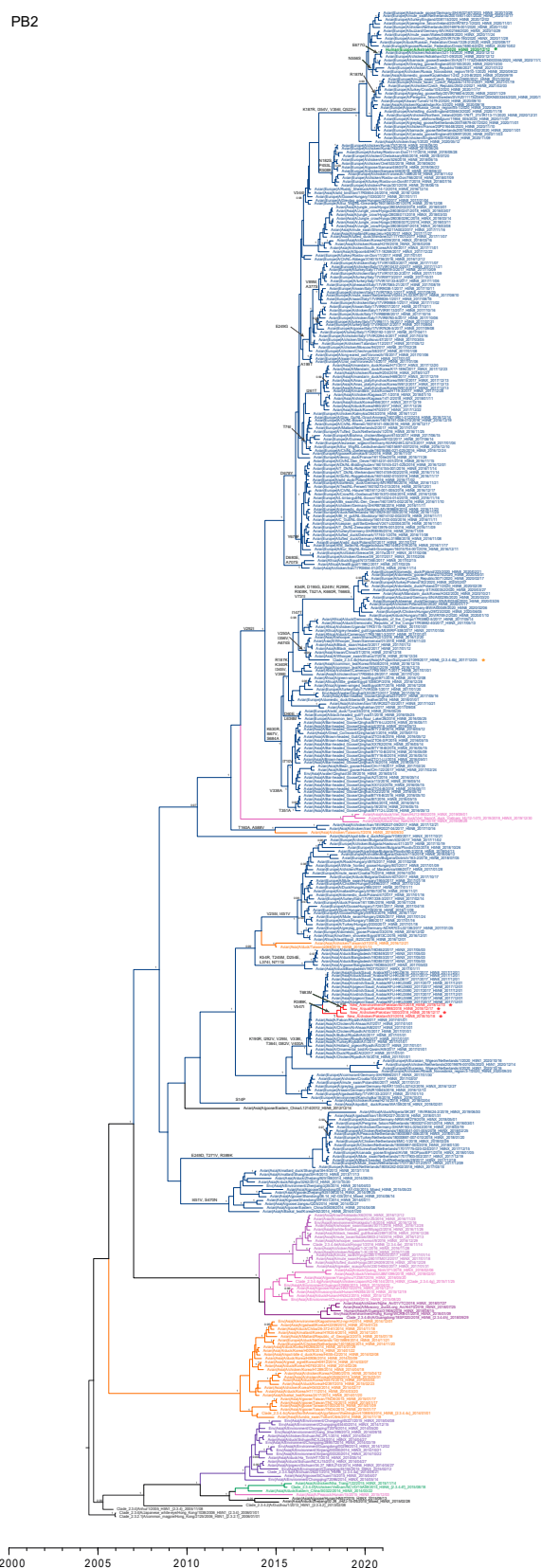
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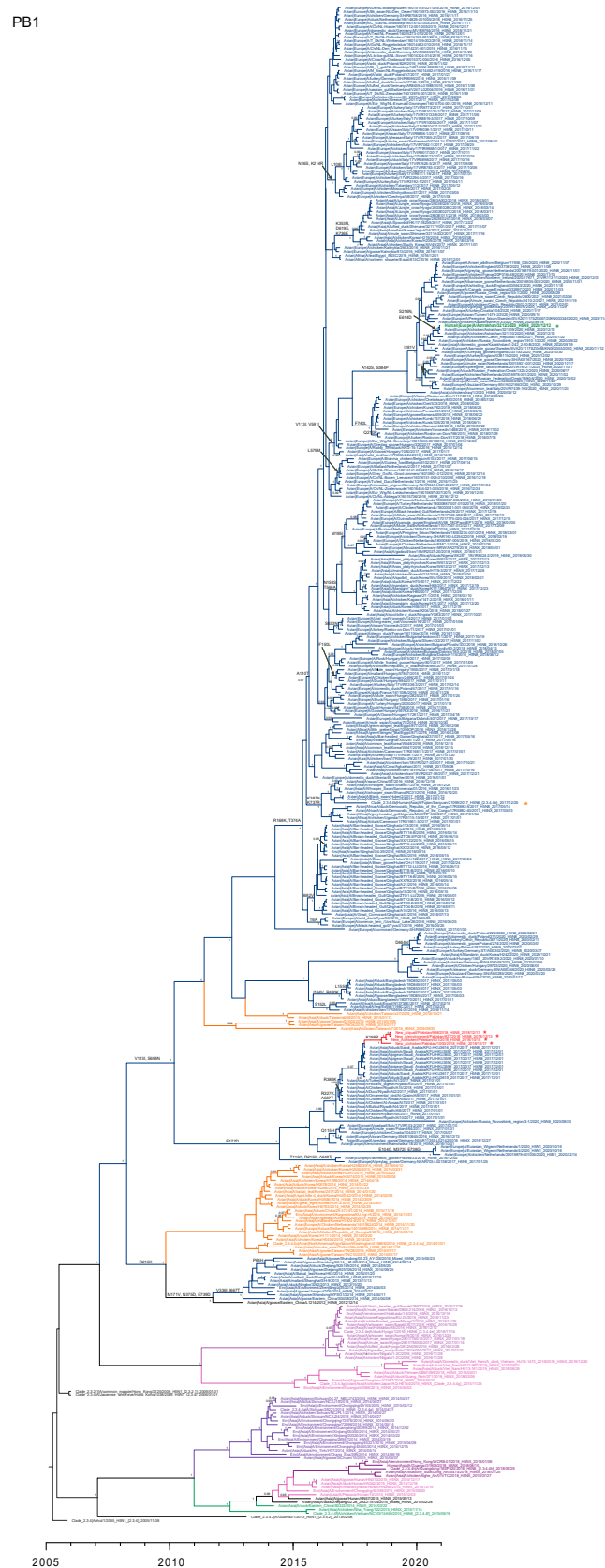
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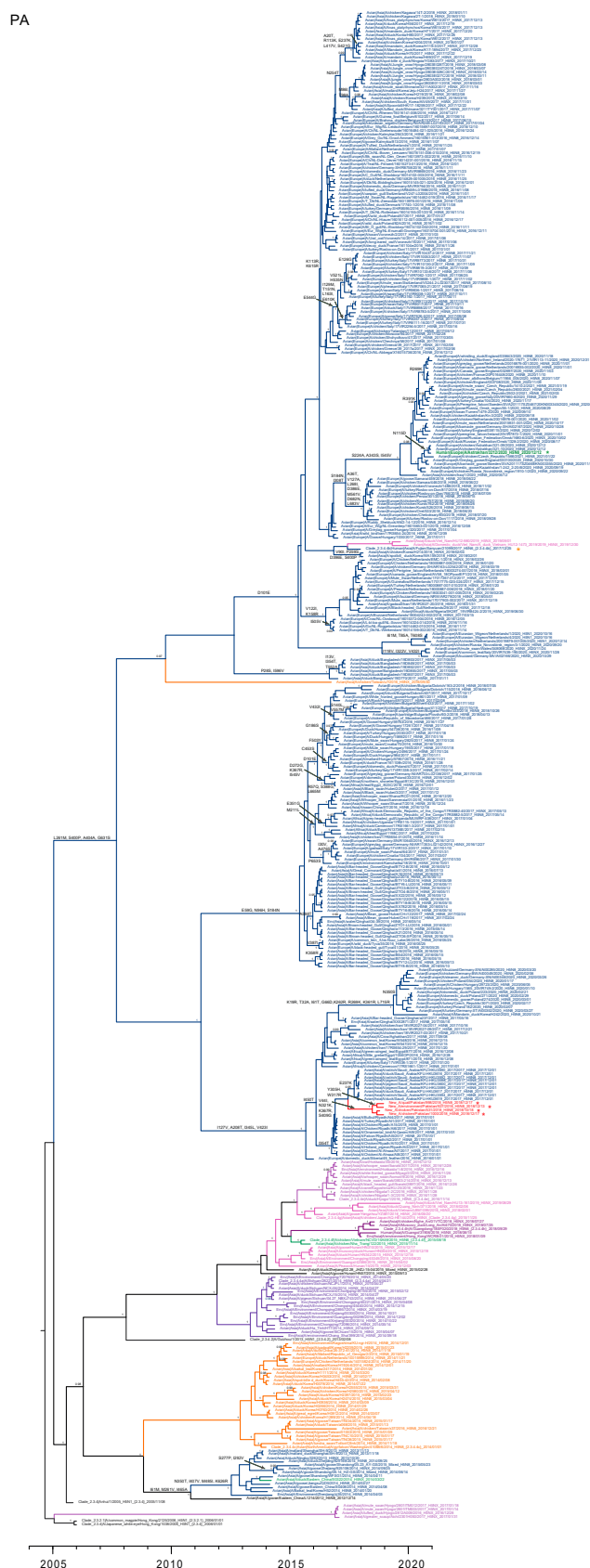
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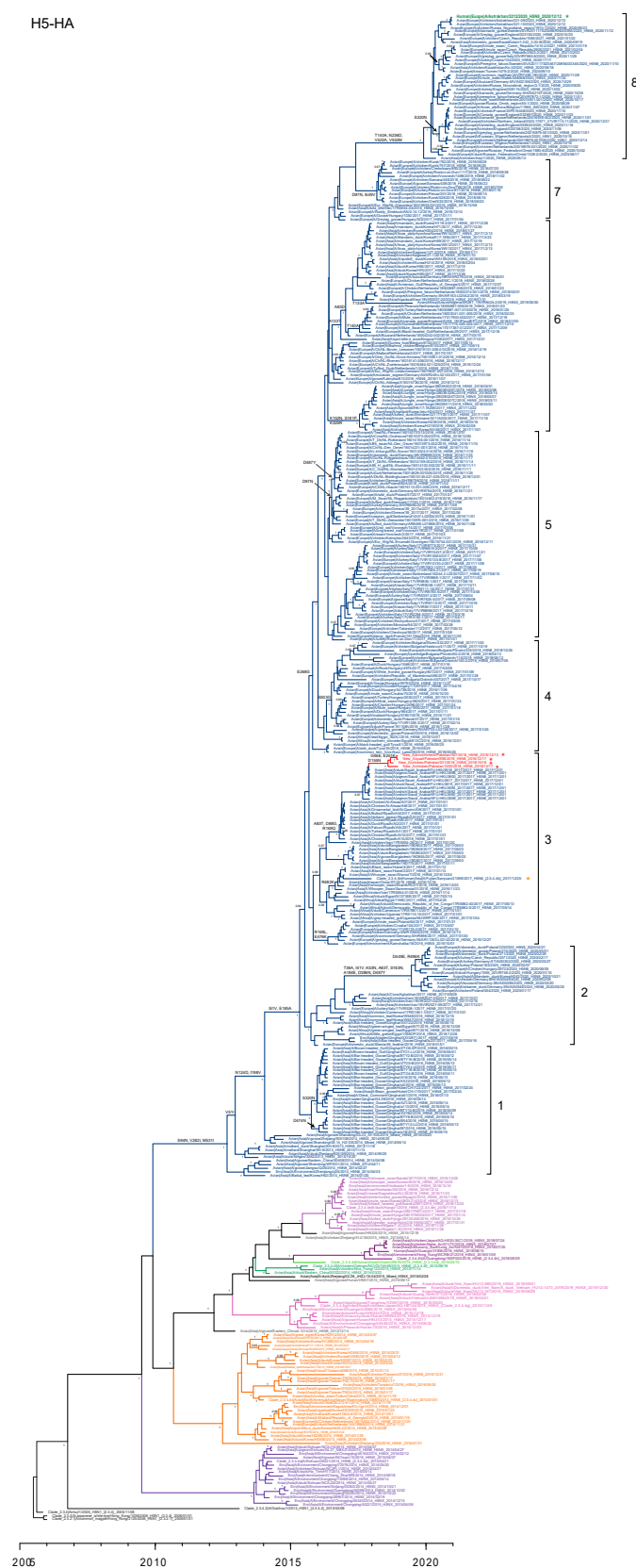
**Figure S1.** Evolutionary relationships of the PB2 gene of highly pathogenic clade 2.3.4.4 A(H5) influenza viruses. Branch colors represent eight different clades of 2.3.4.4 viruses (2.3.4.4a–2.3.4.4h). Red asterisks indicate novel Pakistan A(H5N8) viruses; green asterisk indicates human A(H5N8) virus; and orange asterisk denotes vaccine strain. Significant amino acid mutations are indicated at major nodes. Bayesian posterior probability (PP) values greater than 0.95 are shown.



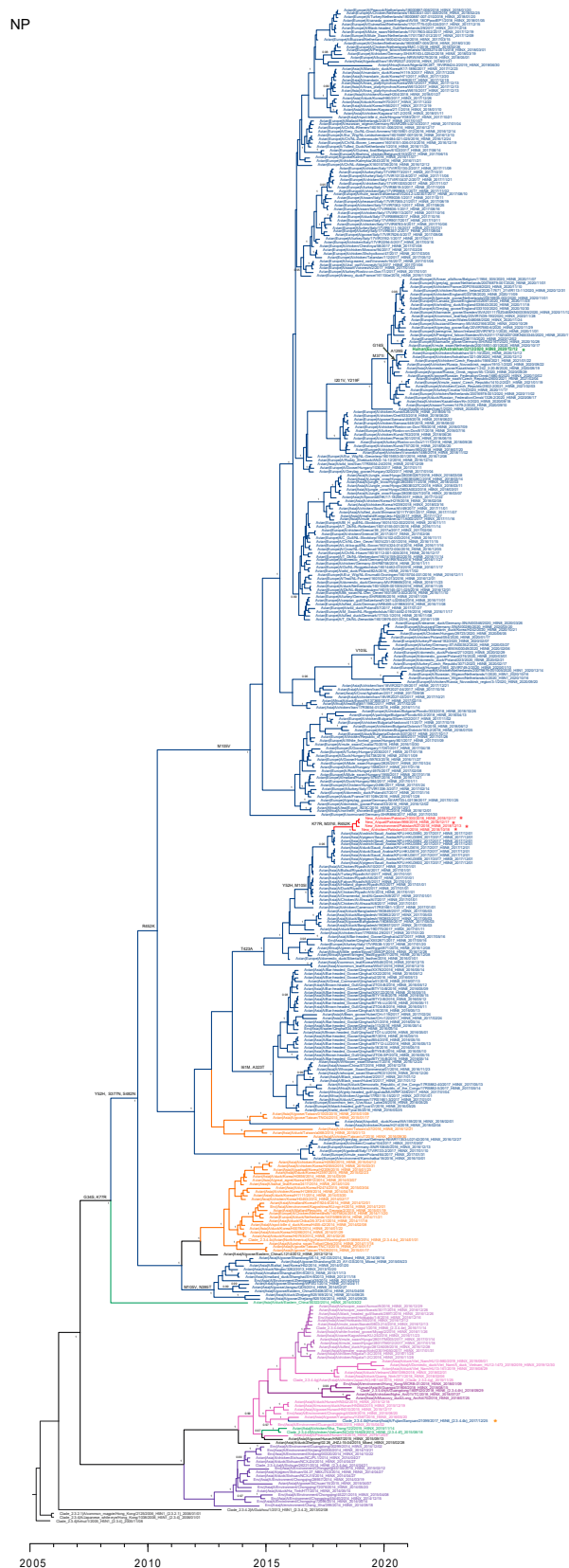
**Figure S2.** Evolutionary relationships of the PB1 gene of highly pathogenic clade 2.3.4.4 A(H5) influenza viruses. Branch colors represent eight different clades of 2.3.4.4 viruses (2.3.4.4a–2.3.4.4h). Red asterisks indicate novel Pakistan A(H5N8) viruses; green asterisk indicates human A(H5N8) virus; and orange asterisk denotes vaccine strain. Significant amino acid mutations are indicated at major nodes. Bayesian posterior probability (PP) values greater than 0.95 are shown.



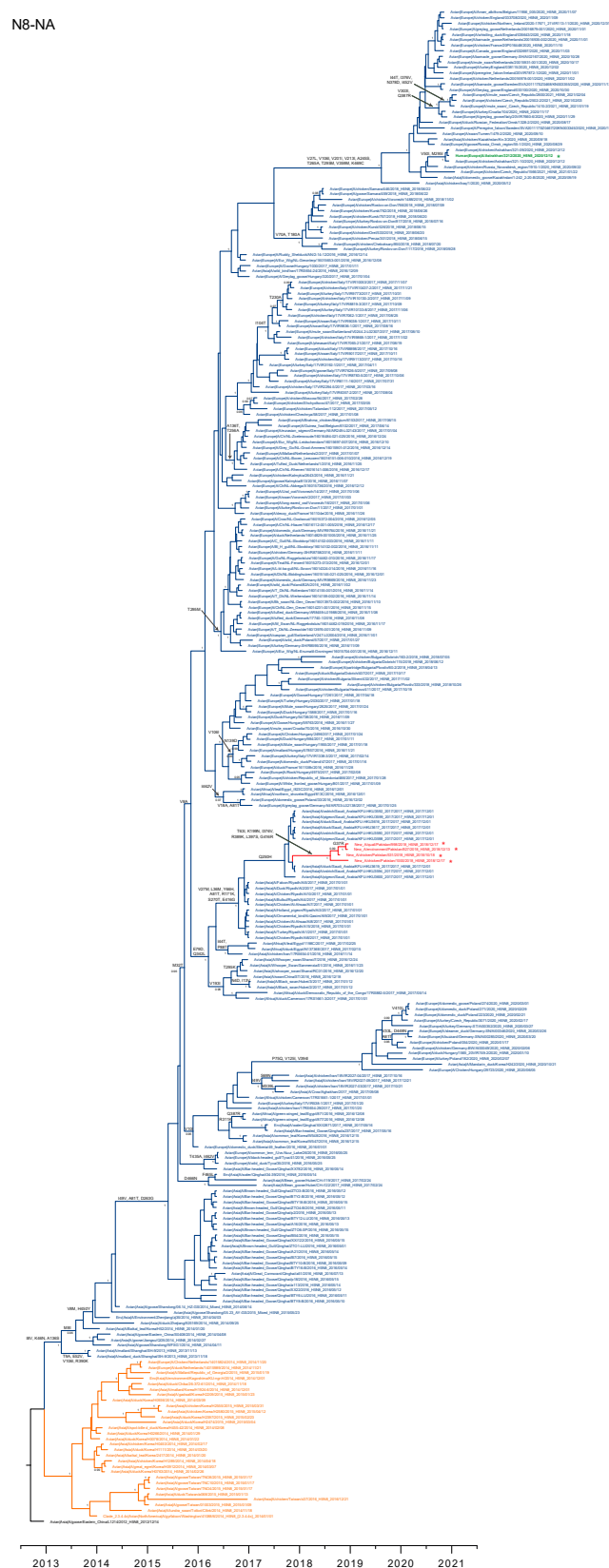
**Figure S3.** Evolutionary relationships of the PA gene of highly pathogenic clade 2.3.4.4 A(H5) influenza viruses. Branch colors represent eight different clades of 2.3.4.4 viruses (2.3.4.4a–2.3.4.4h). Red asterisks indicate novel Pakistan A(H5N8) viruses; green asterisk indicates human A(H5N8) virus; and orange asterisk denotes vaccine strain. Significant amino acid mutations are indicated at major nodes. Bayesian posterior probability (PP) values greater than 0.95 are show.



**Figure S4.** Evolutionary relationships of the H5-HA gene of highly pathogenic clade 2.3.4.4 A(H5) influenza viruses. Branch colors represent eight different clades of 2.3.4.4 viruses (2.3.4.4a–2.3.4.4h). Red asterisks indicate novel Pakistan A(H5N8) viruses; green asterisk indicates human A(H5N8) virus; and orange asterisk denotes vaccine strain. Significant amino acid mutations are indicated at major nodes. Bayesian posterior probability (PP) values greater than 0.95 are shown.

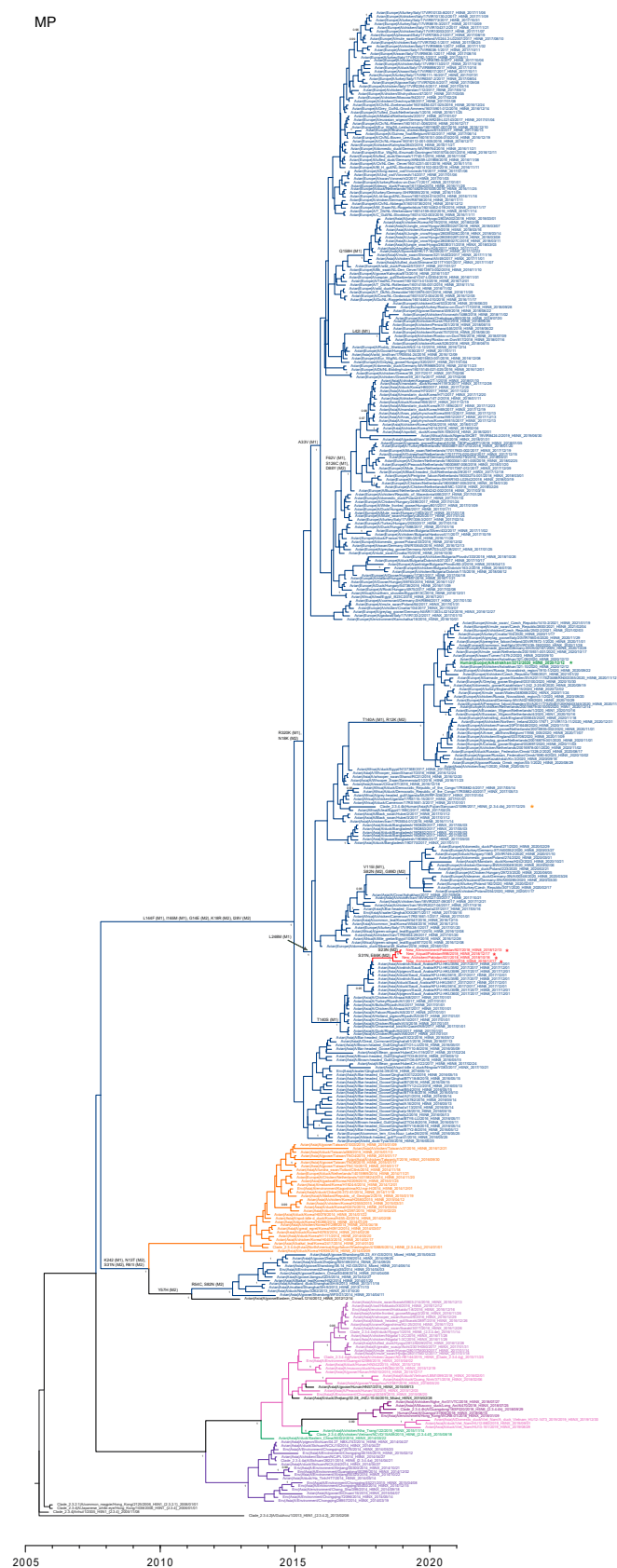


**Figure S5.** Evolutionary relationships of the NP gene of highly pathogenic clade 2.3.4.4 A(H5) influenza viruses. Branch colors represent eight different clades of 2.3.4.4 viruses (2.3.4.4a–2.3.4.4h). Red asterisks indicate novel Pakistan A(H5N8) viruses; green asterisk indicates human A(H5N8) virus; and orange asterisk denotes vaccine strain. Significant amino acid mutations are indicated at major nodes. Bayesian posterior probability (PP) values greater than 0.95 are shown.



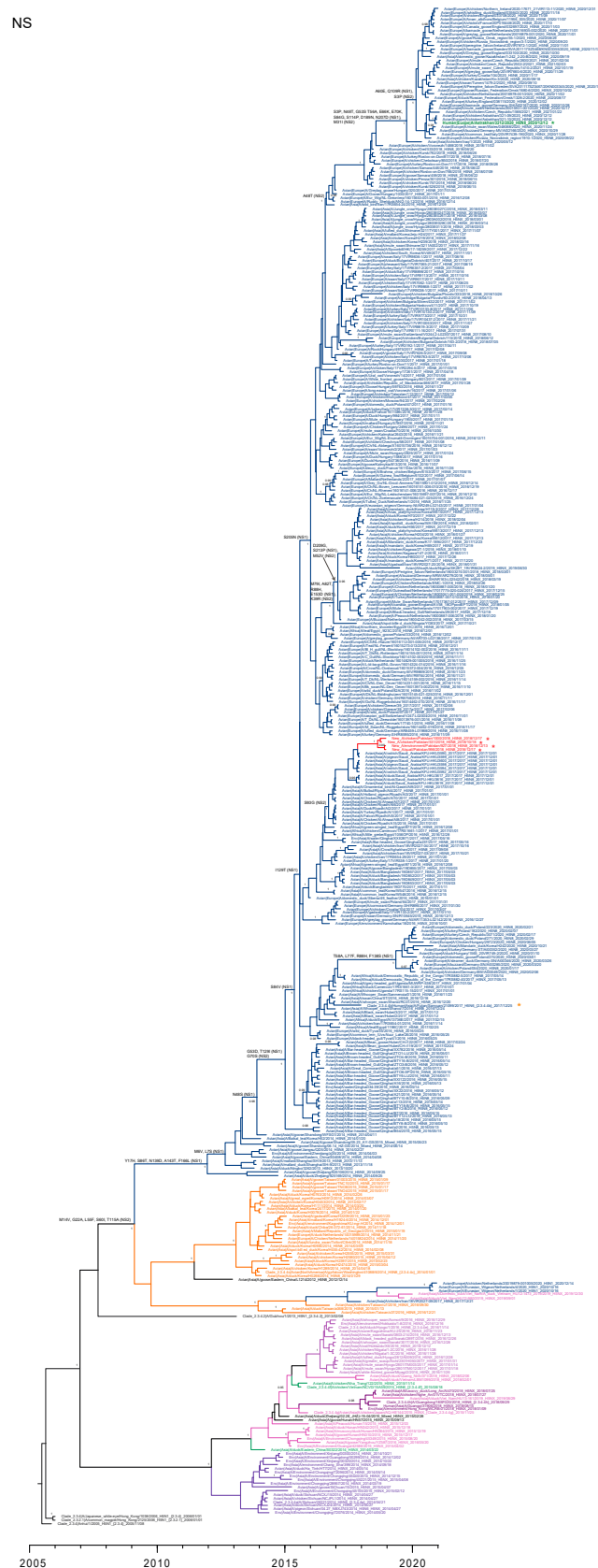
**Figure S6.** Evolutionary relationships of the N8-NA gene of highly pathogenic clade 2.3.4.4 A(H5) influenza viruses. Branch colors represent eight different clades of 2.3.4.4 viruses (2.3.4.4a–2.3.4.4h). Red asterisks indicate novel Pakistan A(H5N8) viruses; green asterisk indicates human A(H5N8) virus; and orange asterisk denotes vaccine strain. Significant amino acid mutations are indicated at major nodes. Bayesian posterior probability (PP) values greater than 0.95 are shown.





**Figure S7.** Evolutionary relationships of the MPgene of highly pathogenic clade 2.3.4.4 A(H5) influenza viruses. Branch colors represent eight different clades of 2.3.4.4 viruses (2.3.4.4a–2.3.4.4h). Red asterisks indicate novel Pakistan A(H5N8) viruses; green asterisk indicates human A(H5N8) virus; and orange asterisk denotes vaccine strain. Significant amino acid mutations are indicated at major nodes. Bayesian posterior probability (PP) values greater than 0.95 are shown.





**Figure S8.** Evolutionary relationships of the NS gene of highly pathogenic clade 2.3.4.4 A(H5) influenza viruses. Branch colors represent eight different clades of 2.3.4.4 viruses (2.3.4.4a–2.3.4.4h). Red asterisks indicate novel Pakistan A(H5N8) viruses; green asterisk indicates human A(H5N8) virus; and orange asterisk denotes vaccine strain. Significant amino acid mutations are indicated at major nodes. Bayesian posterior probability (PP) values greater than 0.95 are shown.