



中国动物卫生与流行病学中心
China Animal Health And Epidemiology Center

Molecular Epidemiology and Phylogenetics

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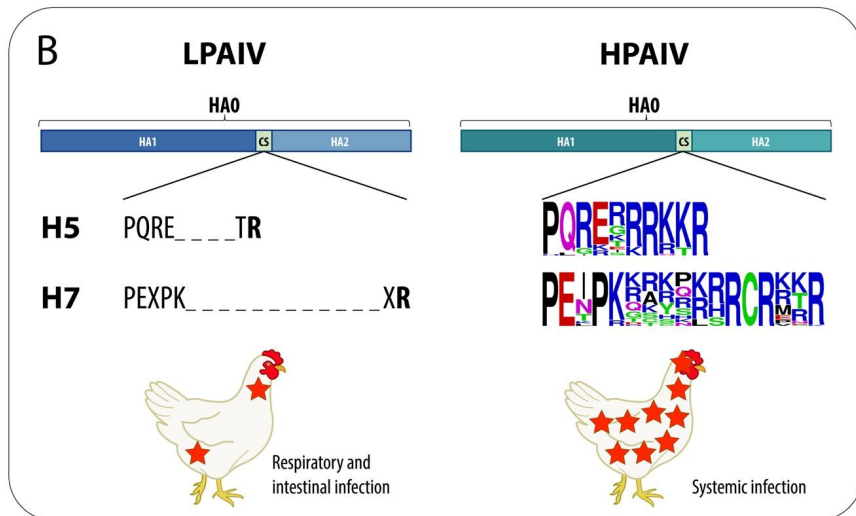
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Avian Diseases Surveillance Laboratory
China Animal Health and Epidemiology Center



Requirements analysis Survey

- Laboratory molecular detection methods
- RT-PCR, Sanger sequencing of the amplification product
- Masterhigh throughput sequencing (Illumina sequencing, Nanopore sequencing)
- Browse and log in to the public database (NCBI, GISAID et al.)
- Sequence identification: blast against GenBank/GISAID, identification of gene segment and subtype
- Download sequences from public databases
- Bioinformatics software
 - Alignment: MAFFT, MUSCLE, Clustal W
 - Phylogenetic tree construction: IQ-TREE, RAxML, MEGA, BEAST
 - Visualization: FigTree, iTOL, ggtree
- Nomenclature systems (e.g. H5 Gs/GD HPAI lineage) standardize classification
- Phylogenetics data analysis: traces transmission pathways
- Do you apply genetic evolution analysis in your work?



- ## ***Key molecular markers for functional annotation***

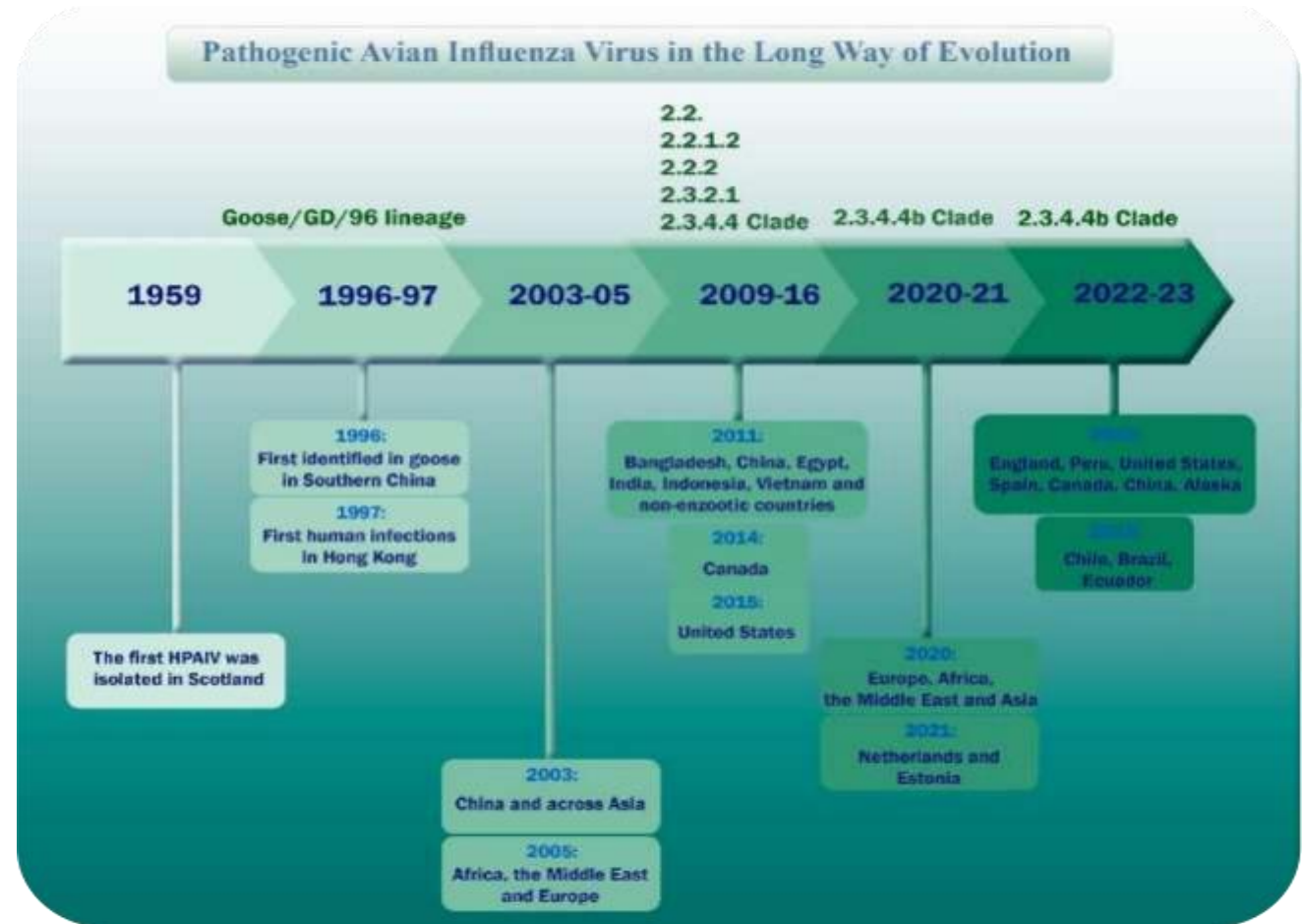
- Molecular pathotyping of H5/H7
 - Based on determination of the cleavage site sequence of the HA0 precursor
 - HPAI (≥ 4 basic amino acids) vs LPAI (< 4 basic amino acids)
- Receptor-binding sites: Q226L and G228S (H3 numbering)
- Utilizes the full genome for comparison, with the ability of fine resolution comparison of more closely related
- **NA:** stalk deletion (associated with adaptation)
- **Internal gene**
 - PB2: E627K, D701N (mammalian adaptation)
 - M2: S31N (amantadine resistance markers)



Brief history of H5 HPAIV

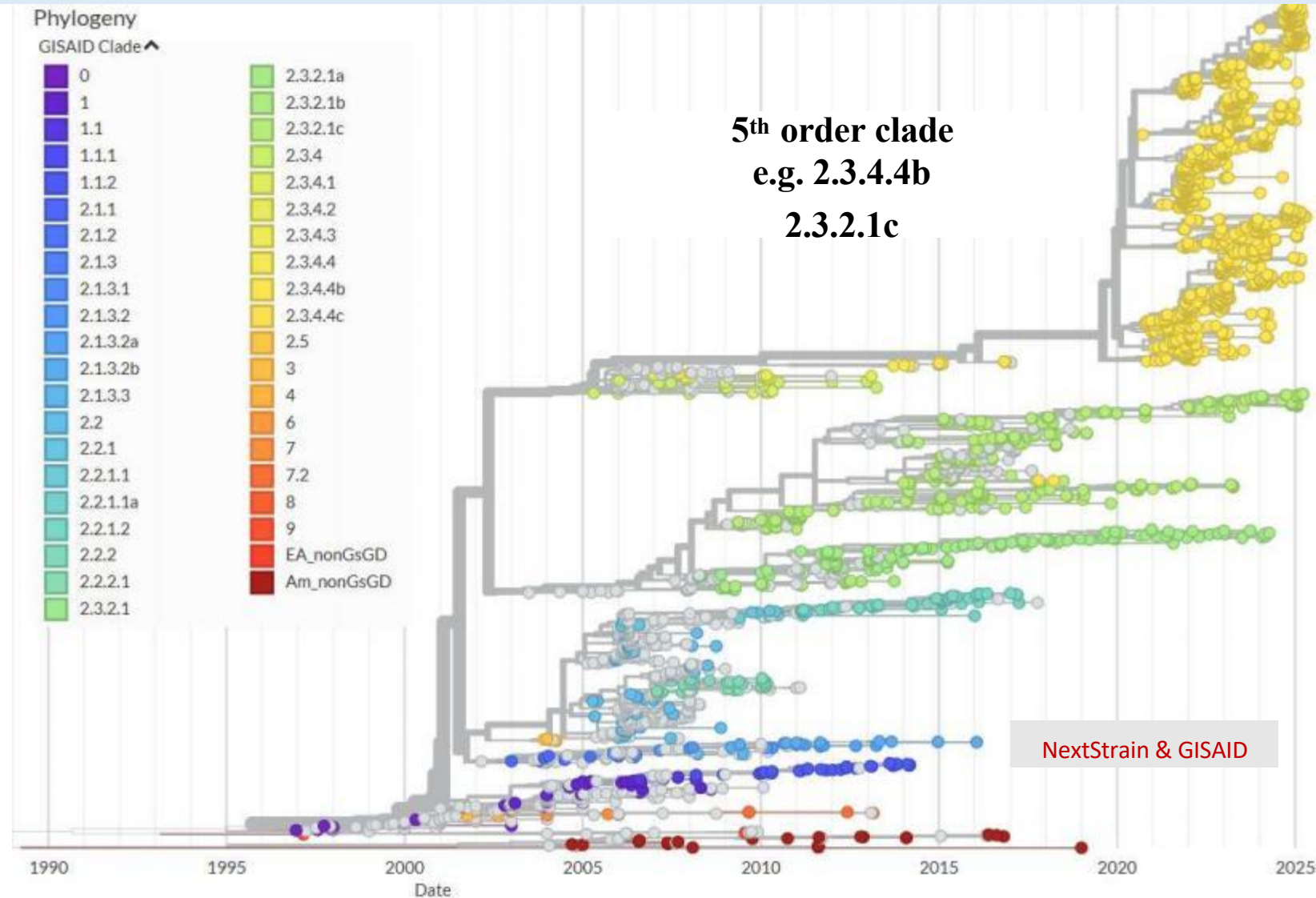
A/Goose/Guangdong/1996 - A lineage that went global

- H5N1 avian influenzavirus isolated in a goose in Guangdong - 1996
- Marked the **birth of the Gs/GD lineage**
- Multiple transcontinental waves over the past two decades driven by wild birds and amplified by poultry trade
- **Waves expanding further geographically**
- **>130 countries** have been affected by H5 HPAI viruses [Gs/GD/96 lineage] since 2003
- Long-standing endemicity in some areas



HPAI - an ever-changing, persistent, global threat

- Extensive mutation (drift) in the HA gene in the past two decades
- Gs/GD/96 gave rise to **multiple genetic clades/subclades** (e.g. predominant clade 2.3.4.4b)
- Clade 0, 3, 4, 5, 6, 7, 8, 9, and several second- and third-order groups have not been detected for many years.





Evolution of H5 Hemagglutinin

Unified nomenclature system for H5 HPAI by the WHO/WOAH/FAO H5N1 Evolution Working Group

■ Based on phylogenetic tree topology

■ Root

- Rooted at the clade 0 node (GS/Guangdong/1/96 lineage)
- This provides the reference for all downstream clade designations

■ Clade designation criteria

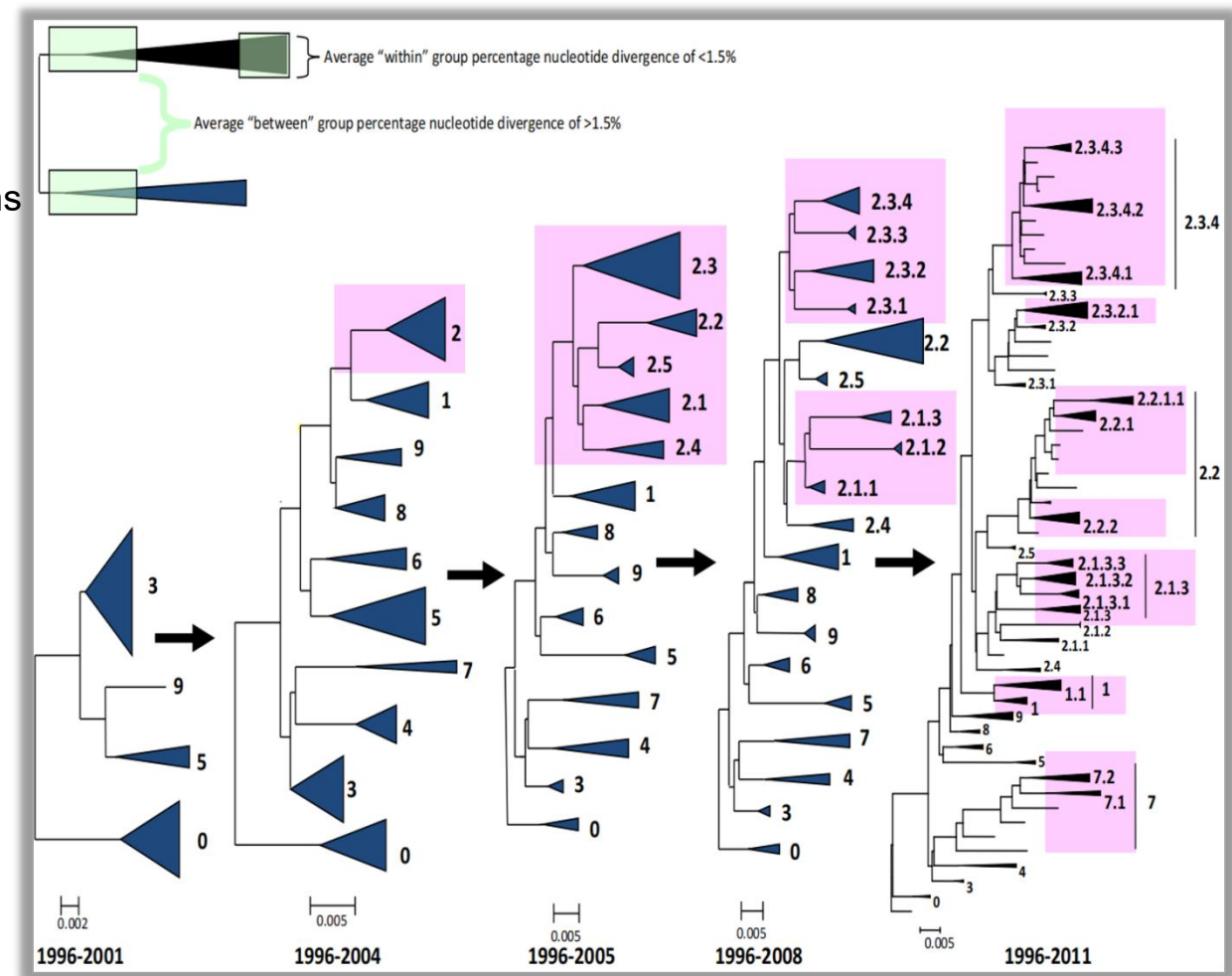
- Distinct common node shared by at least 4 isolates
- Monophyletic grouping with a bootstrap value ≥ 60 at the clade-defining node (after 1000 neighbor-joining bootstrap replicates)

■ Genetic distance threshold

- Between-clade distance $\geq 1.5\%$
- Within-clade distance $\leq 1.5\%$

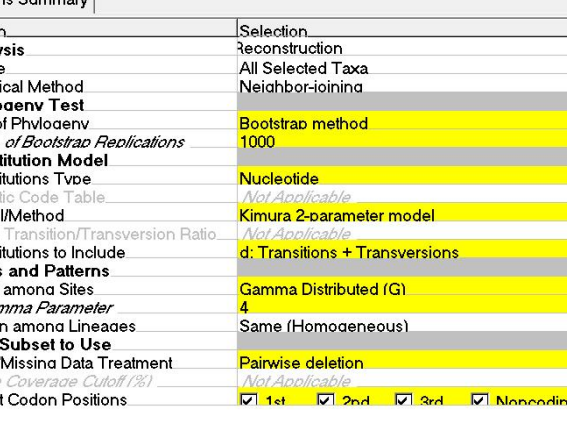
■ Hierarchical expansion of clades

- First order $\rightarrow$ Second order $\rightarrow$ Third order $\rightarrow$ Additional orders
- Clade $\rightarrow$ Subclade $\rightarrow$ Sub-subclade $\rightarrow$ Additional orders
- Based on sharing of common nodes





- Export: File → Export (Newick for iTOL, PNG/PDF for PPT)



Option	Selection
Analysis	Reconstruction
Scope	All Selected Taxa
Statistical Method	Neighbor-joining
Phyloenenv Test	
Test of Phyloenenv	Bootstrap method
<i>No. of Bootstrap Replications</i>	1000
Substitution Model	
Substitutions Type	Nucleotide
Genetic Code Table	Not Applicable
Model/Method	Kimura 2-parameter model
Fixed Transition/Transversion Ratio	Not Applicable
Substitutions to Include	d: Transitions + Transversions
Rates and Patterns	
Rates among Sites	Gamma Distributed (G)
<i>Gamma Parameter</i>	4
Pattern among Lineages	Same (Homogeneous)
Data Subset to Use	
Gaps/Missing Data Treatment	Pairwise deletion
<i>Site Coverage Cutoff (%)</i>	Not Applicable
Select Codon Positions	☒ 1st ☒ 2nd ☒ 3rd ☒ Noncoding Sites

✓ Compute
✗ Cancel
? Help