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## Highly Pathogenic Avian Influenza A(H5N1) Virus in Wild Red Foxes, the Netherlands, 2021

## Appendix 1

## Methods

Phylogenetic analysis was performed for each gene segment as previously described (1). Selected related sequences were obtained from GISAID's EpiFlu database on May 26, 2021 (http://www.gisaid.org) (2). Sequences were aligned using MAFFT v7.427 (3). Maximum likelihood trees based on the general time reversible model with a gamma-distributed variation of rates and 1000 bootstraps were generated using RAxML v8.2.12 (4) and visualized using FigTree 1.4.4 (https://github.com/rambaut/figtree/releases). GISAID accession numbers of the sequences and bootstrap values above 50 are shown in the phylogenetic trees. H5N1 virus sequences originating from the fox cubs are marked in red, high pathogenic avian H5N1 virus sequences from the Netherlands (2020–2021) are marked in green.

## References

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Appendix 1 Figure 1. Phylogenetic tree of PB2 gene segment.



Appendix 1 Figure 2. Phylogenetic tree of PB1 gene segment.



Appendix 1 Figure 3. Phylogenetic tree of PA gene segment.



Appendix 1 Figure 4. Phylogenetic tree of HA gene segment.



Appendix 1 Figure 5. Phylogenetic tree of NP gene segment.



Appendix 1 Figure 6. Phylogenetic tree of NA gene segment.



Appendix 1 Figure 7. Phylogenetic tree of MP gene segment.



Appendix 1 Figure 8. Phylogenetic tree of NS gene segment.