**Virologica Sinica**

**Supplementary Data**

**The evolution, pathogenicity and transmissibility of quadruple reassortant H1N2 swine influenza virus in China: A potential threat to public health**

Xinxin Cui a, b, c, 1, Jinhuan Ma a, b, c, 1, Zifeng Panga, b, c, 1, Lingzhi Chi d, Cuishan Mai a, b, c, Hanlin Liu a, b, c,

Ming Liao a, b, c, e, \*, Hailiang Sun a, b, c, \*

a. College of Veterinary Medicine, South China Agricultural University, Guangzhou, 510642, China

b. Key Laboratory of Zoonosis Control and Prevention of Guangdong Province, South China Agricultural University, Guangzhou, 510642, China

c. National and Regional Joint Engineering Laboratory for Medicament of Zoonosis Prevention and Control, South China Agricultural University, Guangzhou, 510642, China

d. Shandong Vocational Animal Science and Veterinary College, Weifang, 261061, China

e. Institute of Animal Health, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, China

**\* Correspondence:**

Email address: mliao@scau.edu.cn (M. Liao); hsun@scau.edu.cn (H. Sun)

ORCID: 0000-0001-8731-4528 (M. Liao); 0000-0002-3609-4729 (H. Sun)

1 Xinxin Cui, Jinhuan Ma and Zifeng Pang contribute equally to this work

**Supplementary Data**

**Supplementary figure 1**. Phylogenetic trees of *PB2*, *PB1*, *PA*, *NP*, *NS* and *M* genes for swine influenza viruses. The red color indicates human influenza viruses. The red circles indicate the H1N2 isolates in this study.

**Supplementary Table S1. Marginal likelihoods of different combinations of clock model and tree prior.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Segment** | **Molecular clock model** | **Coalescent tree prior** | **Path sampling Marginal L Estimate** | **Stepping-stone Marginal L Estimate** |
| HA | Strict clock | Bayesian skyline | -8385.0063 | -8378.1075 |
| Strict clock | Exponential growth | -8443.4378 | -8438.5175 |
| Strict clock | Constant size | -8565.2833 | -8558.4150 |
| Uncorrelated lognormal relaxed clock | Bayesian skyline | -8328.4687 | -8319.0472 |
| **Uncorrelated lognormal Relaxed clock** | **Exponential growth** | **-8325.4048** | **-8319.0471** |
| Uncorrelated lognormal Relaxed clock | Constant size | -8331.3091 | -8321.3308 |

The best-fitting tree prior and molecular clock model are indicated in bold font.

**Supplementary Table S2. Nucleotide and amino acid homology of five strains.**

**Supplementary Table S2a. Nucleotide and amino acid homology of *HA* gene of five strains**

|  |  |
| --- | --- |
| **Strain name** | **Sequence identities with strains isolated in this study** |
|  | SW/188/20 | SW/198/20 | SW/209/20 | SW/510/21 | SW/700/21 |
| SW/188/20 | - | 91.98 | 96.28 | 95.92 | 96.27 |
| SW/198/20 | 92.48 | - | 94.12 | 93.40 | 93.43 |
| SW/209/20 | 96.11 | 93.63 | - | 99.11 | 99.64 |
| SW/510/21 | 96.00 | 93.36 | 99.65 | - | 99.47 |
| SW/700/21 | 96.06 | 93.53 | 99.82 | 99.82 | - |

The gray shadow part is amino acid homology.

**Supplementary Table S2b. Nucleotide and amino acid homology of *NA* gene of five strains**

|  |  |
| --- | --- |
| **Strain name** | **Sequence identities with strains isolated in this study** |
|  | SW/188/20 | SW/198/20 | SW/209/20 | SW/510/21 | SW/700/21 |
| SW/188/20 | - | 100.00 | 96.66 | 96.68 | 96.49 |
| SW/198/20 | 100.00 | - | 96.65 | 96.88 | 96.70 |
| SW/209/21 | 96.88 | 97.30 | - | 100.00 | 100.00 |
| SW/510/21 | 96.95 | 97.38 | 99.92 | - | 100.00 |
| SW/700/20 | 96.88 | 97.30 | 100.00 | 99.93 | - |

The gray shadow part is amino acid homology.

**Supplementary Table S2c. Nucleotide and amino acid homology of *PB2* gene of five strains**

|  |  |
| --- | --- |
| **Strain name** | **Sequence identities with strains isolated in this study** |
|  | SW/188/20 | SW/198/20 | SW/209/20 | SW/510/21 | SW/700/21 |
| SW/188/20 | - | 99.47 | 99.07 | 99.07 | 99.20 |
| SW/198/20 | 98.94 | - | 98.82 | 98.82 | 98.95 |
| SW/209/21 | 97.41 | 97.41 | - | 100.00 | 99.87 |
| SW/510/21 | 97.95 | 97.50 | 99.91 | - | 99.87 |
| SW/700/20 | 98.14 | 97.63 | 99.52 | 99.60 | - |

The gray shadow part is amino acid homology.

**Supplementary Table S2d. Nucleotide and amino acid homology of *PB1* gene of five strains**

|  |  |
| --- | --- |
| **Strain name** | **Sequence identities with strains isolated in this study** |
|  | SW/188/20 | SW/198/20 | SW/209/20 | SW/510/21 | SW/700/21 |
| SW/188/20 | - | 98.28 | 98.94 | 98.55 | 98.68 |
| SW/198/20 | 97.01 | - | 98.81 | 98.42 | 98.55 |
| SW/209/21 | 97.98 | 98.46 | - | 99.08 | 99.21 |
| SW/510/21 | 97.19 | 97.63 | 98.59 | - | 99.87 |
| SW/700/20 | 97.19 | 97.63 | 98.55 | 99.96 | - |

The gray shadow part is amino acid homology.

**Supplementary Table S2e. Nucleotide and amino acid homology of *NP* gene of five strains**

|  |  |
| --- | --- |
| **Strain name** | **Sequence identities with strains isolated in this study** |
|  | SW/188/20 | SW/198/20 | SW/209/20 | SW/510/21 | SW/700/21 |
| SW/188/20 | - | 100.00 | 94.29 | 94.47 | 94.36 |
| SW/198/20 | 100.00 | - | 94.25 | 94.44 | 94.36 |
| SW/209/21 | 97.30 | 97.56 | - | 99.80 | 99.79 |
| SW/510/21 | 97.36 | 97.62 | 99.93 | - | 100.00 |
| SW/700/20 | 97.26 | 97.53 | 99.87 | 99.93 | - |

The gray shadow part is amino acid homology.

**Supplementary Table S2f. Nucleotide and amino acid homology of *PA* gene of five strains**

|  |  |
| --- | --- |
| **Strain name** | **Sequence identities with strains isolated in this study** |
|  | SW/188/20 | SW/198/20 | SW/209/20 | SW/510/21 | SW/700/21 |
| SW/188/20 | - | 99.30 | 98.74 | 98.88 | 98.88 |
| SW/198/20 | 97.95 | - | 98.33 | 98.47 | 98.47 |
| SW/209/21 | 97.63 | 96.79 | - | 99.86 | 99.86 |
| SW/510/21 | 97.72 | 96.89 | 99.91 | - | 100.00 |
| SW/700/20 | 97.72 | 96.89 | 99.81 | 99.81 | - |

The gray shadow part is amino acid homology.

**Supplementary Table S2g. Nucleotide and amino acid homology of *NS* gene of five strains**

|  |  |
| --- | --- |
| **Strain name** | **Sequence identities with strains isolated in this study** |
|  | SW/188/20 | SW/198/20 | SW/209/20 | SW/510/21 | SW/700/21 |
| SW/188/20 | - | 100.00 | 97.49 | 97.85 | 97.49 |
| SW/198/20 | 100.00 | - | 97.49 | 97.85 | 97.49 |
| SW/209/21 | 97.49 | 98.21 | - | 99.64 | 100.00 |
| SW/510/21 | 97.61 | 98.33 | 99.88 | - | 99.64 |
| SW/700/20 | 97.49 | 98.21 | 100 | 99.88 | - |

The gray shadow part is amino acid homology.

**Supplementary Table S2h. Nucleotide and amino acid homology of *M* gene of five strains**

|  |  |
| --- | --- |
| **Strain name** | **Sequence identities with strains isolated in this study** |
|  | SW/188/20 | SW/198/20 | SW/209/20 | SW/510/21 | SW/700/21 |
| SW/188/20 | - | 100.00 | 99.07 | 97.83 | 98.45 |
| SW/198/20 | 99.69 | - | 99.08 | 97.87 | 98.47 |
| SW/209/21 | 97.96 | 97.26 | - | 98.78 | 99.39 |
| SW/510/21 | 97.04 | 96.44 | 98.88 | - | 99.39 |
| SW/700/20 | 97.14 | 96.53 | 98.98 | 99.70 | - |

The gray shadow part is amino acid homology.

**Supplementary Table S3.** **Genes of the five isolates with the closest nucleotide homology sequence in GenBank.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Strain Name** | **Gene** | **Closest Virus** | **Homology (%)** |
| SW/188/20 | HA | A/swine/Liaoning/PJ89/2014(H1N1) | 97.59% |
|  | NA | A/Guangdong/YueFang277/2017(H3N2) | 99.79% |
|  | PB2 | A/Guangdong/YueFang277/2017(H3N2) | 99.29% |
|  | PB1 | A/swine/Liaoning/CY102/2014(H1N1) | 97.98% |
|  | PA | A/swine/Liaoning/PJ43/2014(H1N1) | 99.21% |
|  | NP | A/Guangdong/YueFang277/2017(H3N2) | 99.94% |
|  | NS | A/Guangdong/YueFang277/2017(H3N2) | 99.40% |
|  | M | A/swine/Hong Kong/2403/2012(H1N1) | 98.47% |
| SW/198/20 | HA | A/swine/Guangdong/3747/2011(H1N1) | 97.00% |
|  | NA | A/Guangdong/YueFang277/2017(H3N2) | 99.36% |
|  | PB2 | A/Guangdong/YueFang277/2017(H3N2) | 99.47% |
|  | PB1 | A/Guangdong/YueFang277/2017(H3N2) | 99.96% |
|  | PA | A/swine/Hong Kong/4893/2012(H3N2) | 98.65% |
|  | NP | A/Guangdong/YueFang277/2017(H3N2) | 99.54% |
|  | NS | A/swine/Liaoning/PJ89/2014(H1N1) | 99.40% |
|  | M | A/swine/Hong Kong/2403/2012(H1N1) | 97.86% |
| SW/209/20 | HA | A/swine/Liaoning/PJ89/2014(H1N1) | 98.05% |
|  | NA | A/swine/Hong Kong/NS3335/2014(H3N2) | 98.30% |
|  | PB2 | A/swine/Guangxi/NNXD2023/2013(H1N1) | 98.29% |
|  | PB1 | A/swine/Liaoning/CY102/2014(H1N1) | 100% |
|  | PA | A/swine/Liaoning/PJ43/2014(H1N1) | 98.23% |
|  | NP | A/swine/Guangxi/BB1/2013(H1N1) | 98.68% |
|  | NS | A/swine/Guangxi/NNXD2023/2013(H1N1) | 98.69% |
|  | M | A/Jiangsu/ALS1/2011(H1N1) | 99.81% |
| SW/510/21 | HA | A/swine/Liaoning/PJ89/2014(H1N1) | 97.94% |
|  | NA | A/swine/Guangdong/2863/2012(H3N2) | 98.37% |
|  | PB2 | A/swine/Guangxi/NNXD2023/2013(H1N1) | 98.38% |
|  | PB1 | A/swine/Liaoning/CY102/2014(H1N1) | 98.59% |
|  | PA | A/swine/Liaoning/PJ43/2014(H1N1) | 98.33% |
|  | NP | A/swine/Guangxi/BB1/2013(H1N1) | 98.75% |
|  | NS | A/swine/Guangxi/NNXD2023/2013(H1N1) | 98.81% |
|  | M | A/swine/Liaoning/CY102/2014(H1N1) | 99.39% |
| SW/700/21 | HA | A/swine/Liaoning/PJ89/2014(H1N1) | 98.00% |
|  | NA | A/swine/Hong Kong/NS3335/2014(H3N2) | 98.30% |
|  | PB2 | A/swine/Guangxi/NNXD2023/2013(H1N1) | 98.77% |
|  | PB1 | A/swine/Liaoning/CY102/2014(H1N1) | 98.59% |
|  | PA | A/swine/Liaoning/PJ43/2014(H1N1) | 98.33% |
|  | NP | A/swine/Liaoning/PJ89/2014(H1N1) | 98.73% |
|  | NS | A/swine/Guangxi/NNXD2023/2013(H1N1) | 98.69% |
|  | M | A/swine/Liaoning/FS487/2015(H1N1) | 99.59% |

Note: Strains marked in red color indicate human influenza viruses.