

months of age and were potentially preventable by MenB vaccine vaccination.

During 2012–13 through 2014–15, a total of 25 (67.5%) of 37 MenW cases in infants were confirmed by culture; 18 (49%) of these cases were phenotypically characterized as MenW:2a, a surrogate phenotypic marker for the hypervirulent ST11 MenW strain. Ten (48%) of the 21 isolates from infants during 2014–15 were MenW:2a, compared with 1 (25%) of 4 during 2012–13 (Figure, panel B). Final diagnoses reported for 20 infants included meningitis (n = 10 [50%]), septicemia (n = 3 [15%]), both meningitis and septicemia (n = 5 [10%]), and septic arthritis (n = 1 [2%]). From 1998–99 through 2014–15, six infants died of MenW IMD (case-fatality rate 3.4%). Four of those deaths occurred during the Hajj outbreak; only 1 death attributed to MenW occurred during the 3 most recent epidemiologic years.

The rapid increase in MenW cases among infants, particularly most recently (2014–15), is cause for concern, and the contemporaneous introduction of MenB vaccine into the national immunization schedule is timely. Although this vaccine is licensed for prevention of MenB disease, the antigens are not specific to this capsular group and could protect against other meningococcal capsular groups that share the same antigens as those in the vaccine. Infants and toddlers immunized with MenB vaccine are expected to develop bactericidal antibodies against ST11 MenW. Data on age distribution suggest that ≈70% of MenW cases in infants could be prevented by MenB vaccination at 2 and 4 months of age. Beginning in mid-2016, the MenB vaccine booster for children 1 year of age is also expected to protect toddlers, for whom MenW cases have also rapidly increased (3).

### Acknowledgments

We appreciate and acknowledge the hard work of the surveillance team at Public Health England Colindale, the laboratory staff at Public Health England's Meningococcal Reference Unit, and local health protection teams.

### References

- Ladhani SN, Giuliani MM, Biolchi A, Pizza M, Beebeejaun K, Lucidarme J, et al. Effectiveness of meningococcal B vaccine against endemic hypervirulent *Neisseria meningitidis* W strain, England. *Emerg Infect Dis*. 2016;22:309–11. <http://dx.doi.org/10.3201/eid2202.150369>
- Ladhani SN, Beebeejaun K, Lucidarme J, Campbell H, Gray S, Kaczmarski E, et al. Increase in endemic *Neisseria meningitidis* capsular group W sequence type 11 complex associated with severe invasive disease in England and Wales. *Clin Infect Dis*. 2015;60:578–85. <http://dx.doi.org/10.1093/cid/ciu881>
- Campbell H, Saliba V, Borrow R, Ramsay M, Ladhani SN. Targeted vaccination of teenagers following continued rapid endemic expansion of a single meningococcal group W clone (sequence type 11 clonal complex), United Kingdom 2015. *Euro Surveill*. 2015;20:21188. <http://dx.doi.org/10.2807/1560-7917.ES2015.20.28.21188>
- Ramsay ME, Andrews NJ, Trotter CL, Kaczmarski EB, Miller E. Herd immunity from meningococcal serogroup C conjugate vaccination in England: database analysis. *BMJ*. 2003;326:365–6. <http://dx.doi.org/10.1136/bmj.326.7385.365>
- Trotter CL, McVernon J, Ramsay ME, Whitney CG, Mulholland EK, Goldblatt D, et al.; SAGE subgroup. Optimising the use of conjugate vaccines to prevent disease caused by *Haemophilus influenzae* type b, *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Vaccine*. 2008;26:4434–45. <http://dx.doi.org/10.1016/j.vaccine.2008.05.073>
- Ladhani SN, Christensen H, Trotter C, Ramsay ME. Indirect impact of an adolescent meningococcal ACWY conjugate vaccine programme in England with and without catch-up: a transmission dynamic model. Presented at: 13th Congress EMGM, European Meningococcal Disease Society; 2015 September 14–17; Amsterdam, the Netherlands. Abstract 5.01. [http://emgm.eu/meetings/emgm2015/EMGM2015\\_programme.pdf](http://emgm.eu/meetings/emgm2015/EMGM2015_programme.pdf)
- Public Health England. Infection report. Invasive meningococcal disease (laboratory reports in England): 2014/2015 annual data by epidemiological year. Health Protection Report. 2015;9(38) [cited 2016 Mar 23]. [https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/498057/hpr3815\\_imd-crrect.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/498057/hpr3815_imd-crrect.pdf)

Address for correspondence: Shamez Ladhani, Public Health England, Immunisation, Hepatitis and Blood Safety Department, 61 Colindale Ave, London NW9 5EQ, UK; email: shamez.ladhani@phe.gov.uk

## Novel Reassortant Avian Influenza A(H5N6) Viruses in Humans, Guangdong, China, 2015

Yong-Yi Shen,<sup>1</sup> Chang-Wen Ke,<sup>1</sup> Qian Li,<sup>1</sup> Run-Yu Yuan, Dan Xiang, Wei-Xin Jia, Yun-Di Yu, Lu Liu, Can Huang, Wen-Bao Qi, Reina Sikkema, Jie Wu, Marion Koopmans, Ming Liao

Author affiliations: Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, Guangzhou (Y.-Y. Shen); College of Veterinary Medicine, South China Agricultural University, Guangzhou, China (Y.-Y. Shen, Q. Li, W.-X. Jia, Y.-D. Yu, W.-B. Qi, M. Liao); Guangdong Provincial Center for Disease Control and Prevention, Guangzhou (C.-W. Ke, R.-Y. Yuan, J. Wu); Shantou University Medical College, Shantou, China (D. Xiang, L. Liu, C. Huang); Key Laboratory of Veterinary Vaccine Innovation of the Ministry of Agriculture, Guangzhou (W.-B. Qi); National Institute of Public Health and the Environment, Bilthoven, the Netherlands (R. Sikkema, M. Koopmans); National and Regional Joint Engineering Laboratory for Medication of Zoonosis Prevention and Control, Guangzhou (M. Liao)

DOI: <http://dx.doi.org/10.3201/eid2208.160146>

<sup>1</sup>These authors contributed equally to this article.

**To the Editor:** Avian influenza A(H5N6) influenza viruses have circulated among poultry in southern (Jiangxi, Guangdong) and western (Sichuan) provinces of China since 2013 (1,2). In 2014, outbreaks of H5N6 virus infection occurred among poultry in China, Laos, and Vietnam (1). In April 2014, the first case of highly pathogenic H5N6 infection among humans was detected in Sichuan Province (3); the second case was detected on December 3, 2014, in Guangdong Province (4). In December 2015, 4 humans in Guangdong Province were infected with H5N6 influenza (5,6).

To study the genetic basis of continuing human infections with this avian influenza subtype, we sequenced the complete genomes of 2 of the 4 human H5N6 isolates obtained in December 2015 in Guangdong Province. We compared these sequences with those of 1 H6N6 and 8 H5N6 influenza viruses isolated from birds in live poultry markets in this region during 2013–2015 (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/22/8/16-0146-TechApp1.pdf>) and other published genomes of H5, H6N6, and H9N2 avian influenza viruses (online Technical Appendix Table). Phylogenetic analyses of the hemagglutinin (HA) genes showed that all human H5N6 isolates belonged to clade 2.3.4.4 (online Technical Appendix Figure 1, panel A). HA and neuraminidase (NA) genes of some H5N6 viruses isolated in Guangdong Province during 2013–2014 were in the Sichuan-like lineage, but all of those from 2015 were in the Jiangxi-like lineage (online Technical Appendix Figure 1, panels A, B).

Despite the similarities of the HA and NA genes, the 6 internal genes from the 2 human isolates from 2015, A/Guangdong/ZQ874/2015 (H5N6) and A/Guangdong/SZ872/2015 (H5N6) were different from 2 human H5N6 isolates from 2014, A/Sichuan/26221/2014 (H5N6) and A/Guangzhou/39715/2014 (H5N6). The polymerase basic (PB) 2 gene from isolate A/Guangdong/ZQ874/2015 (H5N6) appears to have derived from an H6N6 virus isolated from a duck; all other genes in this isolate were derived from H5N6 viruses that have been circulating among poultry since 2013 (online Technical Appendix Figure 1, panel C; online Technical Appendix Figure 2). This isolate showed high nucleotide identity to 6 of the 8 genes (HA, 96.5%; NA, 98.2%; nucleoprotein (NP), 98.5%; polymerase acidic (PA), 98.3%; PB1, 98.1%; PB2, 98.4%) of the isolate A/chicken/Guangdong/FG594/2015 (H5N6); the identities of the matrix (M) and nonstructural protein (NSP) genes were 76.2% and 79.8% similar, respectively. This finding suggests that undetected reassortants of H5N6 may exist. The other human isolate, A/Guangdong/SZ872/2015 (H5N6), showed high nucleotide identity with A/Yunnan/0127/2015 (H5N6), an isolate collected from a person in Yunnan Province (GenBank accession nos. KT963053–60; online Technical Appendix Table), for all 8 genes (HA, 97.2%; M, 97.7%; NA, 96.8%; NP, 98.3%; NSP, 93.2%; PA, 95.9%; PB1, 96.9%; PB2, 94.0%). The 6 internal genes of A/Guangdong/SZ872/2015 (H5N6) appear to have come from the enzootic H9N2 (ZJ-HJ/07) virus lineage (online Technical Appendix Figure 1, panel C). These

**Table.** Molecular analyses of 5 influenza A(H5N6) virus isolates from humans in China, 2014 and 2015\*

Phenotypic consequences	Mutations	A/Sichuan/ 26221/2014	A/Guangzhou/ 39715/2014	A/Yunan/ 0127/2015	A/Guangdong/ SZ872/2015	A/Guangdong/ ZQ874/2015
<b>HA gene</b>						
Altered receptor specificity	S128P	T	P	P	P	P
Increased $\alpha$ 2,6-SA recognition	S137A	A	A	A	A	A
Removal of the 158N glycosylation	T160A	A	T	A	A	A
Cleavage site sequence	Q226L	Q	Q	Q	Q	Q
	Not applicable	REKRRKR↓G	REKRRKR↓G	REKRRKR↓G	REKRRKR↓G	REKRRKR↓G
<b>NA gene</b>						
59–69 del	TIINNHPQNNF	No	Yes	Yes	Yes	Yes
Osetamivir resistance	H274Y N294S	H N	H N	H N	H N	H N
<b>PB2</b>						
Increased pathogenicity in mice	L89V E627K	V E	V K	V K	V E	V E
Increased virulence and replication in mice	G309D, T339K R477G, I495V	DMGV	DTGV	DKGV	DKGV	DKGV
Enhanced transmission	D701N	N	D	D	D	N
<b>NS1</b>						
Increased virulence in mice and pigs	D92E	E	E	D	D	E
PDZ-motif	Not applicable	ESEV	ESEV	KPEV	KPEV	ESEV
Increased virulence in mice	P42S	S	S	S	S	S
<b>M2</b>						
Antiviral resistance (amantadine)	S31N	S	S	N	N	S

\*HA, hemagglutinin; M2, matrix protein 2; NA, neuraminidase; NS1, nonstructural protein 1; PB2, polymerase basic 2 amino acid.

findings show that the circulating H5N6 virus in southern China has reassorted with enzootic H6N6 and H9N2 viruses, resulting in new H5N6 viruses that are capable of infecting humans.

We compared the 2 newly sequenced genomes with 3 available genomes of human influenza virus strains in public databases to determine if they had attained key molecular features associated with increased virulence in mammals, mammalian transmissibility, and antiviral resistance (Table). The HA gene cleavage site encoded by all 5 isolates from humans showed a multiple basic amino acid motif (REKRRKR↓G), which indicates high pathogenicity in poultry. The viruses isolated from humans in 2014 had no mutations associated with reduced sensitivity to adamantane antiviral drugs, but 2 of the 3 viruses isolated in 2015 have the 31N amino acid in M2, suggesting that those 2 viruses have acquired resistance. Thus, this virus lineage could be a great threat to public health.

Although H9N2 is not highly pathogenic in poultry, it provides internal genes for the recent emergence of many novel avian influenza viruses that infect humans, such as the H5N6 virus in this study, as well as the H7N9 (7,8) and H10N8 (9) viruses. Infection with H6 subtype viruses results in no clinically significant signs of disease in poultry (10), but co-circulation of these viruses with other subtypes among poultry results in transfer of internal genes. This reassortment has resulted in a major increase in genetic diversity among the H5N6 viruses that cause human infections; therefore, low-pathogenicity viruses in poultry should also be controlled in poultry.

In summary, we isolated 2 novel reassortant H5N6 viruses from 2 patients in Guangdong Province, China. The internal genes of these strains are different from those found in the first wave of H5N6 infections in 2014. The PB2 of 2 human isolate A/Guangdong/ZQ874/2015 (H5N6) appears to have been derived from a duck H6N6 virus, and all other genes of this virus originated in circulating H5N6 viruses. In contrast, the 6 internal genes of the other human isolate, A/Guangdong/SZ872/2015 (H5N6), were derived from enzootic H9N2 viruses. Although human infection has been sporadic, the co-circulation and reassortment of this virus with other enzootic low pathogenicity influenza viruses has resulted in new reassortant viruses. Further surveillance of birds is needed to monitor the spread of this novel virus.

This study was supported by the National Natural Science Foundation of China (U1501212); Guangdong Natural Science Funds for Distinguished Young Scholar (2014A030306046), was a Key Project of the Agricultural Ministry (CARS-42-G09), and the Modern Agriculture Talents Support Program (2012, no. 160).

## References

- Shen H, Wu B, Chen Y, Bi Y, Xie Q, Influenza A (H5N6) virus reassortant, Southern China, 2014. *Emerg Infect Dis*. 2015;21:1261–2. <http://dx.doi.org/10.3201/eid2107.140838>
- Qi X, Cui L, Yu H, Ge Y, Tang F. Whole-genome sequence of a reassortant H5N6 avian influenza virus isolated from a live poultry market in China, 2013. *Genome Announc*. 2014;2:e00706–14. <http://dx.doi.org/10.1128/genomeA.00706-14>
- World Health Organization. WHO China statement on H5N6. 7 May 2014 [cited 2016 Mar 28]. <http://www.wpro.who.int/china/mediacentre/releases/2014/20140507/en/>
- Mok CKP, Da Guan W, Liu XQ, Lamers MM, Li XB, Wang M, et al. Genetic characterization of highly pathogenic avian influenza A(H5N6) virus, Guangdong, China. *Emerg Infect Dis*. 2015;21:2268–71. <http://dx.doi.org/10.3201/eid2112.150809>
- World Health Organization. Human infection with avian influenza A(H5N6) virus—China. 04 Jan 2016 [cited 2016 Mar 28]. <http://www.who.int/csr/don/4-january-2016-avian-influenza-china/en/>
- World Health Organization. Human infection with avian influenza A(H5N6) virus—China. 11 Jan 2016 [cited 2016 Mar 28]. <http://www.who.int/csr/don/11-january-2016-avian-influenza-china/en/>
- Lam TT, Zhou B, Wang J, Chai Y, Shen Y, Chen X, et al. Dissemination, divergence and establishment of H7N9 influenza viruses in China. *Nature*. 2015;522:102–5. <http://dx.doi.org/10.1038/nature14348>
- Lam TT-Y, Wang J, Shen Y, Zhou B, Duan L, Cheung C-L, et al. The genesis and source of the H7N9 influenza viruses causing human infections in China. *Nature*. 2013;502:241–4. <http://dx.doi.org/10.1038/nature12515>
- Chen H, Yuan H, Gao R, Zhang J, Wang D, Xiong Y, et al. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. *Lancet*. 2014;383:714–21. [http://dx.doi.org/10.1016/S0140-6736\(14\)60111-2](http://dx.doi.org/10.1016/S0140-6736(14)60111-2)
- Zhao G, Lu X, Gu X, Zhao K, Song Q, Pan J, et al. Molecular evolution of the H6 subtype influenza A viruses from poultry in eastern China from 2002 to 2010. *Virology*. 2011;8:470. <http://dx.doi.org/10.1186/1743-422X-8-470>

Address for correspondence: Ming Liao, College of Veterinary Medicine, South China Agricultural University, Guangzhou, China; email: [mliao@scau.edu.cn](mailto:mliao@scau.edu.cn)

**govDELIVERY** 

**Manage your email alerts so you only receive content of interest to you.**

Sign up for an online subscription:  
[wwwnc.cdc.gov/eid/subscribe.htm](http://wwwnc.cdc.gov/eid/subscribe.htm)

# Novel Reassortant Avian Influenza A(H5N6) Viruses in Humans, Guangdong, China, 2015

## Technical Appendix

### Virus Isolation and Sequencing

On December 28, 2015, avian influenza A(H5N6) was detected in a throat swab sample from a patient (female, 26 years of age) with severe pneumonia by Shenzhen Municipal Center for Disease Control and Prevention (CDC). On December 31, 2015, another H5N6–positive case was detected in throat swab and tracheal aspirate samples from a patient (female, 40 years of age) with fever by Zhaoqing Municipal CDC. Both viruses were further confirmed to be H5N6 by full genome sequencing conducted at Guangdong Provincial CDC. Both H5N6–infected patients died; their deaths were reported to National Health and Family Planning Commission (NHFPC) of the People’s Republic of China and World Health Organization (WHO) (<http://www.who.int/csr/don/11-january-2016-avian-influenza-china/en/>) (<http://www.who.int/csr/don/4-january-2016-avian-influenza-china/en/>).

Long–term avian influenza virus (AIV) surveillance was conducted in Guangdong province. We collected pairs of cloacal and oropharyngeal swab specimens from healthy poultry in several live poultry markets. Samples were stored in viral medium at 4°C until they were transported to the laboratory and then stored at –80°C until virus isolation. A 0.22–µm filter was used to sterilize the samples, which were then used to inoculate 9-day-old SPF embryonated eggs. After incubation at 37 °C for 72 h, allantoic fluid was harvested and tested by using the hemagglutination assay. Subtypes of the viruses were determined by conventional hemagglutination

inhibition and neuraminidase inhibition assays. Nine samples that tested positive for H5 and N6 were chosen for RNA extraction and sequencing in this study.

Complete genomes of the H5N6 and H6N6 AIVs were amplified by using a Qiagen OneStep RT-PCR Kit (Qiagen, Germany). PCR products were purified and sequenced on an ABI 3730XL automatic DNA analyzer. All 8 gene segments were sequenced; the nucleotide sequences were deposited into the Global Initiative on Sharing Avian Influenza Data (GISAID) (accession numbers: EPI\_ISL\_205959-EPI\_ISL\_206569).

## **Data Source and Analyses**

All previously published H5, H6N6, and H9N2 AIV subtype sequences were collated from GenBank and the Global Initiative on Sharing Avian Influenza Data (GISAID) on 3 January 2016. Sequences of each gene segment were aligned by using MUSCLE (1). Phylogenetic trees were constructed by using RAxML (2), and the 94 sequences (Technical Appendix Table) most closely related to the H5N6 outbreak isolates were selected for further refined analyses. Maximum likelihood phylogenies were reconstructed by using PhyML with bootstrap analysis (1,000 replicates) (3). Evolution of virus strains is shown in online Technical Appendix Figure 2. Phylogenetic trees constructed for the HA, NA, polymerase basic-2, polymerase basic-1 (PB1), polymerase acidic (PA), nucleoprotein (NP), matrix (M), and nonstructural (NS) segments are shown in online Technical Appendix Figure 1.

## **References**

1. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 2004;32:1792–7.  
<http://dx.doi.org/10.1093/nar/gkh340>

2. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*. 2006;22:2688–90.  
<http://dx.doi.org/10.1093/bioinformatics/btl446>
3. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol*. 2010;59:307–21. <http://dx.doi.org/10.1093/sysbio/syq010>

**Technical Appendix Table.** Complete genomes of 94 avian influenza viruses isolated from poultry and the environment, China\*

Strain name	Genbank accession numbers							
	PB2	PB1	PA	HA	NP	NA	M	NSP
A/Anas crecca/Hubei/Chenhu1623-5/2014 (H5N6)	KM251532	KM251522	KM251512	KM251462	KM251492	KM251485	KM251472	KM251502
A/black chicken/Jiangxi/10129/2014 (H5N6)	KP286450	KP286451	KP286452	KP286453	KP286454	KP286455	KP286456	KP286457
A/cat/Sichuan/SC18/2014 (H5N6)	KM873635	KM873636	KM873637	KM873638	KM873639	KM873640	KM873641	KM873642
A/chicken/DaLi/302/2014 (H5N1)	KP732559	KP732560	KP732561	KM392379	KP732562	KP732563	KP732564	KP732565
A/chicken/Dongguan/2690/2013 (H5N6)	KP286098	KP286099	KP286100	KP286101	KP286102	KP286103	KP286104	KP286105
A/chicken/Dongguan/3056/2013 (H9N2)	KP414562	KP414563	KP414564	KP414565	KP414566	KP414567	KP414568	KP414569
A/chicken/Dongguan/3363/2013 (H5N6)	KP286106	KP286107	KP286108	KP286109	KP286110	KP286111	KP286112	KP286113
A/chicken/Dongguan/3964/2013 (H9N2)	KP414642	KP414643	KP414644	KP414645	KP414646	KP414647	KP414648	KP414649
A/chicken/Dongguan/4189/2013 (H9N2)	KP414666	KP414667	KP414668	KP414669	KP414670	KP414671	KP414672	KP414673
A/chicken/Dongguan/4259/2013 (H5N6)	KP285026	KP285027	KP285028	KP285029	KP285030	KP285031	KP285032	KP285033
A/chicken/Hong Kong/NT266/2012 (H9N2)	KF260899	KF260655	KF260411	KF259157	KF259923	KF259559	KF259404	KF260167
A/chicken/Hubei/2014 (H9N2)	KT164848	KT164849	KT164850	KT164851	KT164852	KT164853	KT164854	KT164855
A/chicken/Jiangxi/13240/2014 (H10N6)	KP286346	KP286347	KP286348	KP286349	KP286350	KP286351	KP286352	KP286353
A/chicken/Jiangxi/29117/2013 (H9N2)	KP285298	KP285299	KP285300	KP285301	KP285302	KP285303	KP285304	KP285305
A/chicken/Jiangxi/34682/2013 (H9N2)	KP285458	KP285459	KP285460	KP285461	KP285462	KP285463	KP285464	KP285465
A/chicken/Jiangxi/NCDZT1123/2014 (H5N6)	KP090436	KP090437	KP090438	KP090439	KP090440	KP090441	KP090442	KP090443
A/chicken/Jiangxi/NCDZT1126/2014 (H5N6)	KP090444	KP090445	KP090446	KP090447	KP090448	KP090449	KP090450	KP090451
A/chicken/Laos/LPQ001/2014 (H5N6)	KM496962	KM496963	KM496964	KM496965	KM496966	KM496967	KM496968	KM496969
A/chicken/Qingdao/013/2014 (H9N2)	KT449724	KT449704	KT449684	KT449584	KT449644	KT449624	KT449604	KT449664
A/chicken/Rizhao/55/2013 (H9N2)	KF260941	KF260697	KF260453	KF259166	KF259965	KF259569	KF259446	KF260209
A/chicken/Shanghai/SC387/2013 (H9N2)	KM113050	KM113051	KM113052	KM113053	KM113054	KM113055	KM113056	KM113057
A/chicken/Shenzhen/1061/2013 (H5N6)	KP286082	KP286083	KP286084	KP286085	KP286086	KP286087	KP286088	KP286089
A/chicken/Shenzhen/1395/2013 (H5N6)	KP284970	KP284971	KP284972	KP284973	KP284974	KP284975	KP284976	KP284977
A/chicken/Shenzhen/1488/2013 (H9N2)	KP414378	KP414379	KP414380	KP414381	KP414382	KP414383	KP414384	KP414385
A/chicken/Shenzhen/1799/2013 (H9N2)	KP414426	KP414427	KP414428	KP414429	KP414430	KP414431	KP414432	KP414433
A/chicken/Shenzhen/1845/2013 (H5N6)	KP284978	KP284979	KP284980	KP284981	KP284982	KP284983	KP284984	KP284985
A/chicken/Shenzhen/2269/2013 (H5N6)	KP286090	KP286091	KP286092	KP286093	KP286094	KP286095	KP286096	KP286097
A/chicken/Shenzhen/552/2013 (H5N6)	KP284962	KP284963	KP284964	KP284965	KP284966	KP284967	KP284968	KP284969
A/chicken/Shenzhen/715/2013 (H5N6)	KP286074	KP286075	KP286076	KP286077	KP286078	KP286079	KP286080	KP286081
A/chicken/Sichuan/NCJPL1/2014 (H5N6)	KM251533	KM251523	KM251513	KM251463	KM251493	KM251486	KM251473	KM251503
A/chicken/Taizhou/TZJF05/2015 (H9N2)	KU143584	KU143541	KU143498	KU143287	KU143412	KU143351	KU143326	KU143455
A/chicken/Wenzhou/YHQL04/2014 (H9N2)	KU143582	KU143539	KU143496	KU143285	KU143410	KU143349	KU143324	KU143453
A/chicken/Wuhan/WHYJ02/2015 (H5N6)	KU143578	KU143535	KU143492	KU143268	KU143403	KU143364	KU143319	KU143449
A/chicken/Zhejiang/6C2/2013 (H5N6)	KJ807774	KJ807775	KJ807776	KJ807777	KJ807778	KJ807779	KJ807780	KJ807781
A/chicken/Zhejiang/727079/2014_H5N2	KU042667	KU042695	KU042724	KU042753	KU042782	KU042811	KU042840	KU042869
A/chicken/Zhejiang/727155/2014 (H5N6)	KU042679	KU042708	KU042737	KU042766	KU042795	KU042824	KU042853	KU042882
A/chicken/Zhejiang/727192/2014 (H9N2)	KU042521	KU042539	KU042557	KU042575	KU042593	KU042611	KU042629	KU042647
A/chicken/Zhejiang/727198/2014 (H9N2)	KU042522	KU042540	KU042558	KU042576	KU042594	KU042612	KU042630	KU042648
A/chicken/Zhejiang/925117/2014 (H9N2)	KU042525	KU042543	KU042561	KU042579	KU042597	KU042615	KU042633	KU042651
A/chicken/Zhejiang/925134/2014 (H9N2)	KU042527	KU042545	KU042563	KU042581	KU042599	KU042617	KU042635	KU042653
A/duck/Dongguan/3069/2013 (H5N6)	KP285018	KP285019	KP285020	KP285021	KP285022	KP285023	KP285024	KP285025
A/duck/Fujian/5474/2007 (H6N6)	CY109863	CY109864	CY109865	CY109866	CY109867	CY109868	CY109869	CY109870
A/duck/Guangdong/GD01/2014 (H5N6)	KJ754142	KJ754143	KJ754144	KJ754145	KJ754146	KJ754147	KJ754148	KJ754149
A/duck/Hunan/S4273/2010 (H6N6)	KJ200892	KJ200891	KJ200890	KJ200885	KJ200888	KJ200887	KJ200886	KJ200889
A/duck/Jiangsu/k1203/2010 (H5N8)	JQ973691	JQ973692	JQ973693	JQ973694	JQ973695	JQ973696	JQ973697	JQ973698
A/duck/Jiangxi/10160/2014 (H5N6)	KP285034	KP285035	KP285036	KP285037	KP285038	KP285039	KP285040	KP285041



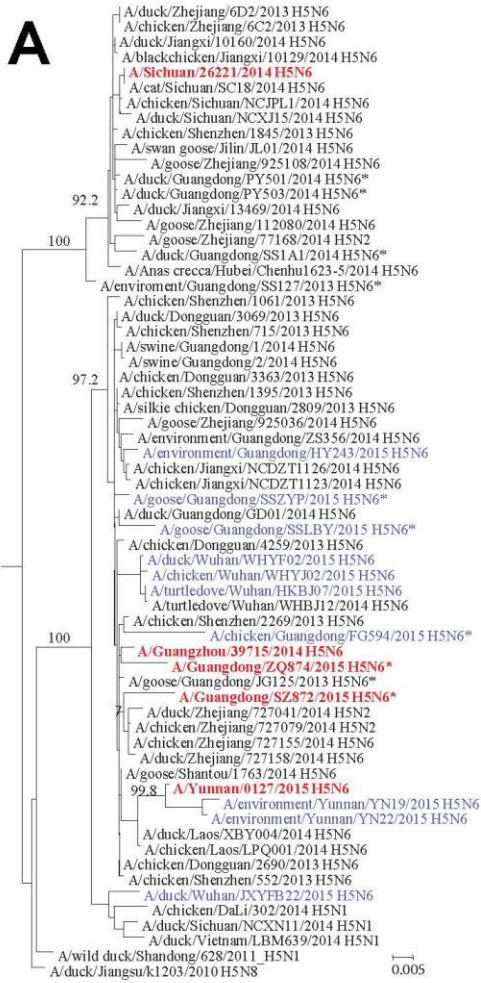
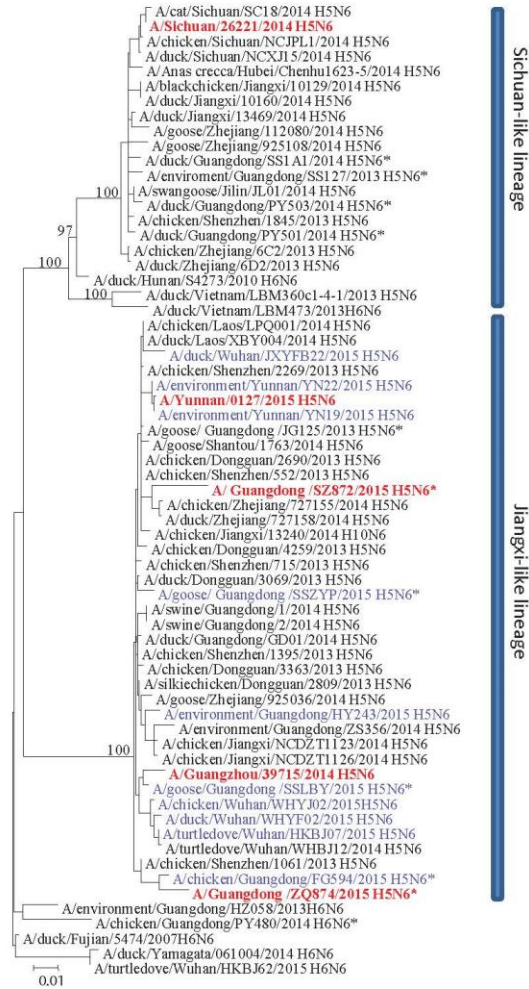
Strain name	Genbank accession numbers							
	PB2	PB1	PA	HA	NP	NA	M	NSP
A/duck/Jiangxi/13469/2014 (H5N6)	KP286418	KP286419	KP286420	KP286421	KP286422	KP286423	KP286424	KP286425
A/duck/Laos/XBY004/2014 (H5N6)	KM496974	KM496975	KM496976	KM496977	KM496978	KM496979	KM496980	KM496981
A/duck/Sichuan/NCXJ15/2014 (H5N6)	KM251535	KM251525	KM251515	KM251465	KM251495	KM251488	KM251475	KM251505
A/duck/Sichuan/NCXN11/2014 (H5N1)	KM251540	KM251530	KM251520	KM251470	KM251500	KM251483	KM251480	KM251510
A/duck/Vietnam/LBM360c1-4-1/2013 (H5N6)	LC010693	LC010694	LC010695	LC010696	LC010697	LC010698	LC010699	LC010700
A/duck/Vietnam/LBM473/2013 (H6N6)	AB916656	AB916657	AB916658	AB916659	AB916660	AB916661	AB916662	AB916663
A/duck/Vietnam/LBM639/2014 (H5N1)	AB979508	AB979509	AB979510	AB979511	AB979512	AB979513	AB979514	AB979515
A/duck/Wenzhou/YHQL64/2014 (H9N2)	KU143583	KU143540	KU143497	KU143286	KU143411	KU143350	KU143325	KU143454
A/duck/Wuhan/JXYFB22/2015 (H5N6)	KU143579	KU143536	KU143493	KU143270	KU143409	KU143368	KU143321	KU143451
A/duck/Wuhan/WHYF02/2015 (H5N6)	KU143575	KU143532	KU143489	KU143265	KU143402	KU143365	KU143318	KU143446
A/duck/Yamagata/061004/2014 (H6N6)	LC042078	LC042079	LC042080	LC042081	LC042082	LC042083	LC042084	LC042085
A/duck/Zhejiang/6D2/2013 (H5N6)	KJ807773	KJ807776	KJ807778	KJ807780	KJ807782	KJ807784	KJ807786	KJ807788
A/duck/Zhejiang/727041/2014 (H5N2)	KU042662	KU042691	KU042720	KU042749	KU042778	KU042807	KU042836	KU042865
A/duck/Zhejiang/727158/2014 (H5N6)	KU042668	KU042697	KU042726	KU042755	KU042784	KU042813	KU042842	KU042871
A/environment/Guangdong/HY243/2015 (H5N6)	KT370112	KT370104	KT370097	KT370063	KT370085	KT370072	KT370070	KT370090
A/environment/Guangdong/HZ058/2013 (H6N6)	KT370052	KT370043	KT370033	KT369984	KT370013	KT370002	KT369994	KT370022
A/environment/Guangdong/ZS356/2014 (H5N6)	KT370107	KT370100	KT370093	KT370061	KT370080	KT370078	KT370065	KT370086
A/environment/Yunnan/YN19/2015 (H5N6)	ND	KT963052	KT963051	KT963046	KT963049	KT963048	KT963047	KT963050
A/environment/Yunnan/YN22/2015 (H5N6)	KT963060	KT963059	KT963058	KT963053	KT963056	KT963055	KT963054	KT963057
A/goose/Shantou/1763/2014 (H5N6)	KP285314	KP285315	KP285316	KP285317	KP285318	KP285319	KP285320	KP285321
A/goose/Zhejiang/112080/2014 (H5N6)	KU042675	KU042704	KU042733	KU042762	KU042791	KU042820	KU042849	KU042878
A/goose/Zhejiang/77168/2014_H5N2	KU042665	KU042694	KU042723	KU042752	KU042781	KU042810	KU042839	KU042868
A/goose/Zhejiang/925036/2014 (H5N6)	KU042671	KU042700	KU042729	KU042758	KU042787	KU042816	KU042845	KU042874
A/goose/Zhejiang/925108/2014 (H5N6)	KU042674	KU042703	KU042732	KU042761	KU042790	KU042819	KU042848	KU042877
A/Guangzhou/39715/2014 (H5N6)	KP765785	KP765786	KP765787	KP765788	KP765789	KP765790	KP765791	KP765792
A/silkie chicken/Dongguan/2809/2013 (H5N6)	KP285010	KP285011	KP285012	KP285013	KP285014	KP285015	KP285016	KP285017
A/swan goose/Jilin/JL01/2014 (H5N6)	KM873643	KM873644	KM873645	KM873646	KM873647	KM873648	KM873649	KM873650
A/swine/Guangdong/1/2014 (H5N6)	KT313409	KT313408	KT313407	KT313418	KT313405	KT313404	KT313403	KT313406
A/swine/Guangdong/2/2014 (H5N6)	KT313417	KT313416	KT313415	KT313410	KT313413	KT313412	KT313411	KT313414
A/turtledove/Wuhan/HKBJ07/2015 (H5N6)	KU143569	KU143527	KU143484	KU143273	KU143408	KU143358	KU143312	KU143440
A/turtledove/Wuhan/HKBJ62/2015 (H6N6)	KU143558	KU143515	KU143472	KU143274	KU143386	KU143356	KU143300	KU143429
A/turtledove/Wuhan/WHBJ12/2014 (H5N6)	KU143570	KU143528	KU143485	KU143261	KU143397	KU143359	KU143313	KU143441
A/wild chicken/Shanghai/C1/2014 (H9N2)	KJ726736	KJ726730	KJ726724	KJ726694	KJ726712	KJ726706	KJ726700	KJ726718
A/wild duck/Shandong/628/2011 (H5N1)	JX534562	JX534563	JX534564	JX534565	JX534566	JX534567	JX534568	JX534569
A/Yunnan/0127/2015 (H5N6)	KT245150	KT245149	KT245148	KT245143	KT245146	KT245145	KT245144	KT245147
A/Hong Kong/308/2014 (H9N2)	EPI498034	EPI498035	EPI498033	EPI498037	EPI498030	EPI498036	EPI498032	EPI498031
A/Sichuan/26221/2014 (H5N6)	EPI533585	EPI533586	EPI533587	EPI533583	EPI533588	EPI533584	EPI533589	EPI533590
A/environment/Guangdong/SS127/2013 (H5N6)†	EPI_ISL_205965							
A/goose/Guangdong /JG125/2013 (H5N6)†	EPI_ISL_205961							
A/goose/Guangdong /SSLBY/2015 (H5N6)†	EPI_ISL_205966							
A/goose/Guangdong /SSZYP/2015 (H5N6)†	EPI_ISL_205967							
A/chicken/Guangdong/FG594/2015 (H5N6)†	EPI_ISL_205964							
A/duck/Guangdong/PY501/2014 (H5N6)†	EPI_ISL_205962							
A/duck/Guangdong/PY503/2014 (H5N6)†	EPI_ISL_205963							
A/duck/Guangdong/SS1A1/2014 (H5N6)†	EPI_ISL_205959							
A/Guangdong/SZ872/2015 (H5N6)†	EPI_ISL_206568							
A/Guangdong/ZQ874/2015 (H5N6)†	EPI_ISL_206569							
A/Chicken/Guangdong/PY480/2014 (H6N6)†	EPI_ISL_205960							

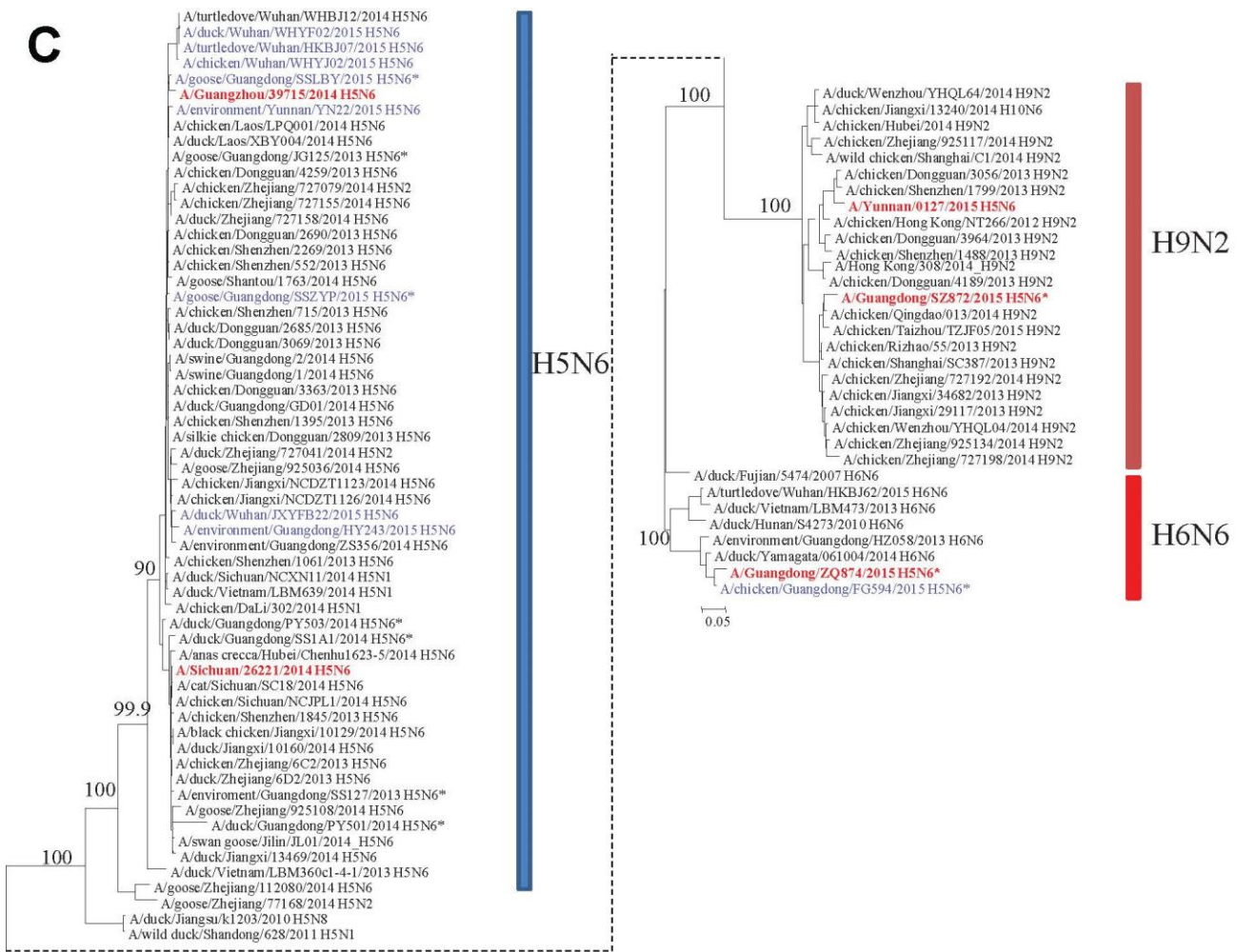


Strain name	Genbank accession numbers							
	PB2	PB1	PA	HA	NP	NA	M	NSP

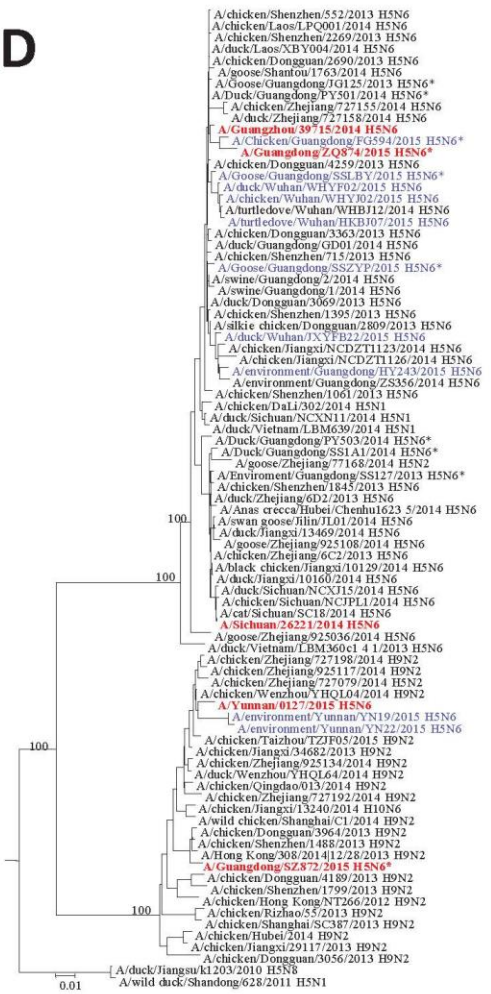
\*PB, polymerase basic; PA, polymerase; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NSP, nonstructural protein; ND, no data.

†The complete genomes of these viruses were deposited in the Global Initiative on Sharing Avian Influenza Data (GISAID) EpiFlu database (<http://platform.gisaid.org>).

**A****B**



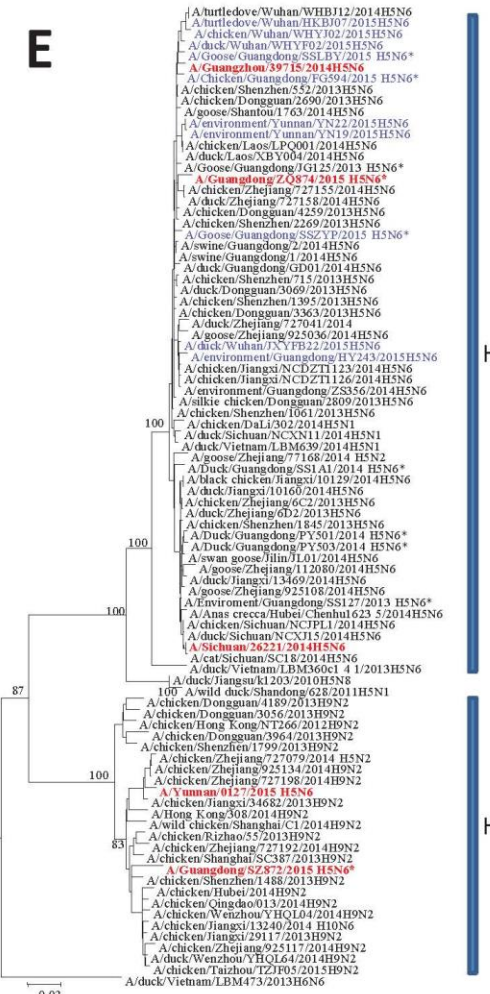
D



H5N6

H9N2

E

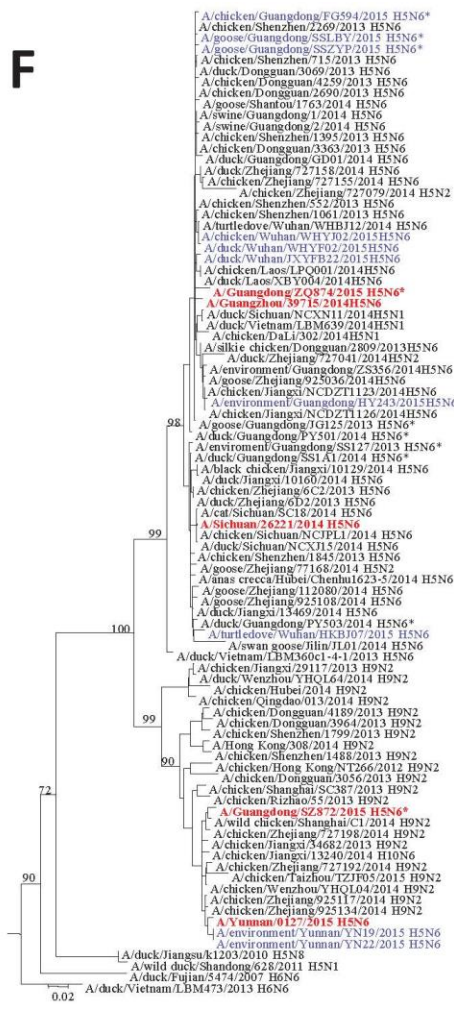


H5N6

H9N2



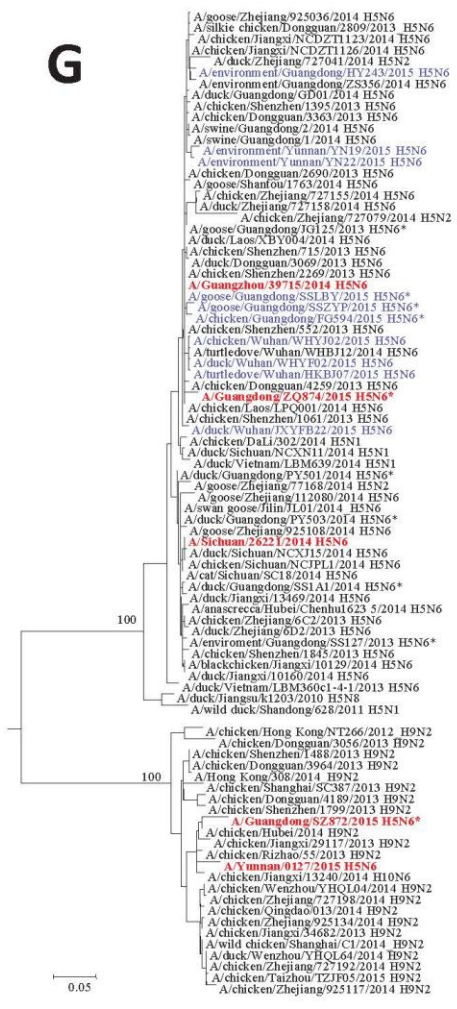
F



G

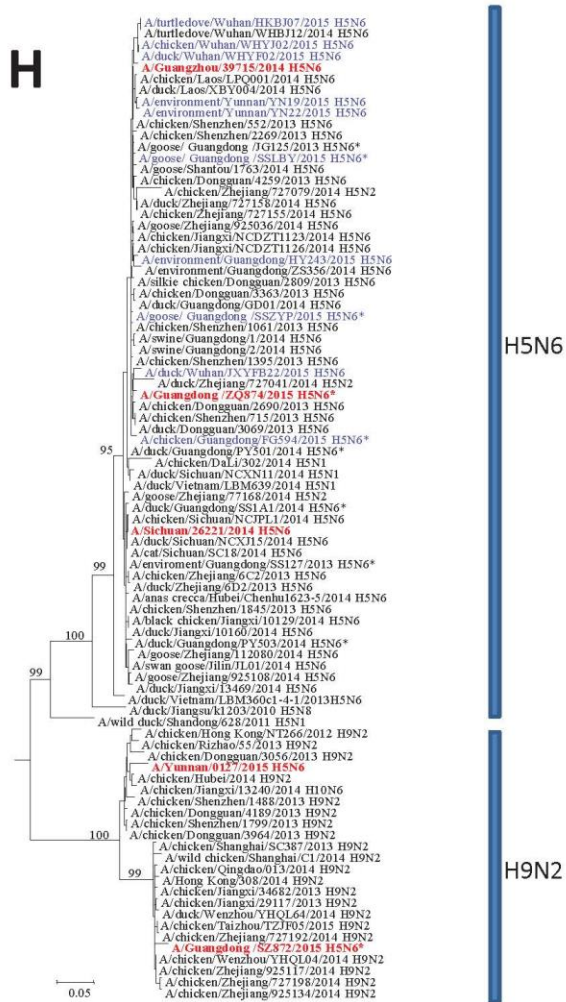
H5N6

H9N2

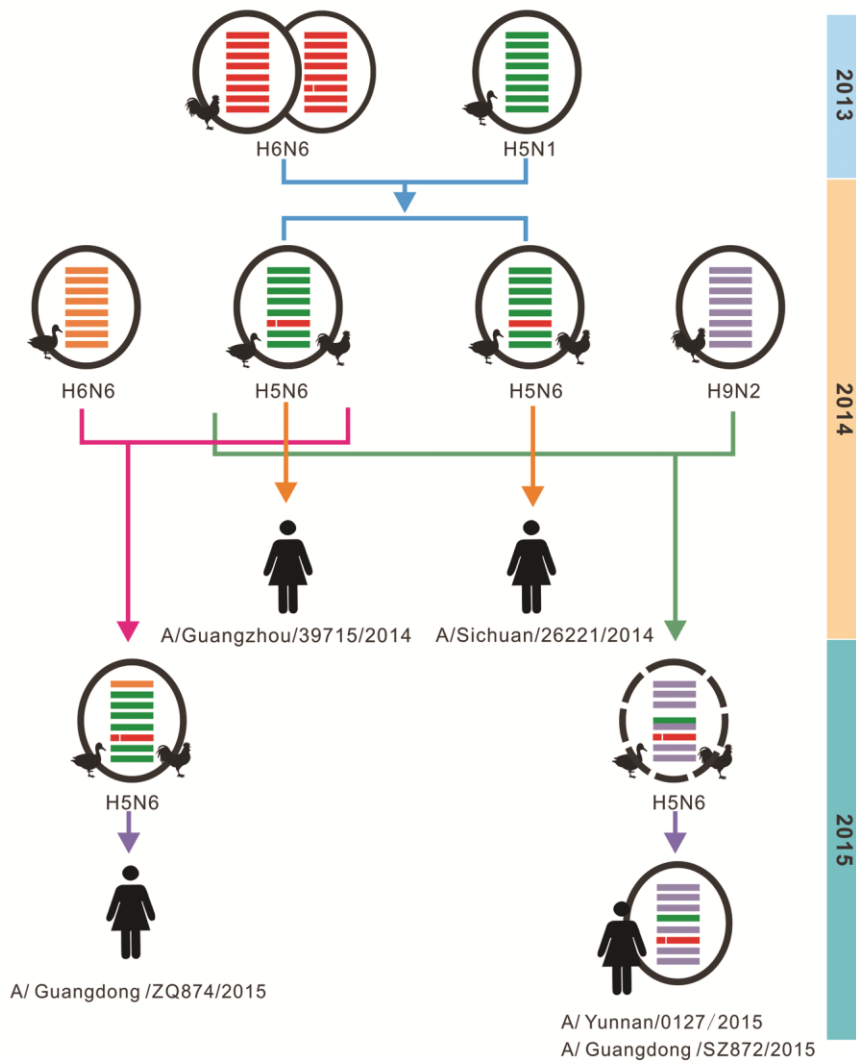


H5N6

H9N2



**Technical Appendix Figure 1.** Phylogenetic relationships of H5N6 influenza viruses. A) hemagglutinin (HA); B) neuraminidase (NA); C) polymerase basic-2 (PB2); D) polymerase basic-1 (PB1); E) polymerase acidic (PA); F) nucleoprotein (NP); G) matrix (M); H) non-structural (NS). Maximum-likelihood trees were constructed using PhyML. Bootstrap values were calculated on 1,000 replicates. Viruses that infect humans are indicated in red. H5N6 viruses isolated in 2015 are indicated in blue; viruses sequenced in this study were marked with \*.



**Technical Appendix Figure 2.** Hypothetical lineage for the origin of the novel reassortant avian influenza A(H5N6) viruses in humans, Guangdong, China, 2015. Virus particles are represented by ovals containing horizontal bars that represent the 8 gene segments (from top to bottom: polymerase basic 2, polymerase basic 1, polymerase acidic protein, hemagglutinin, nucleocapsid protein, neuraminidase, matrix protein, and nonstructural protein). A broken bar representing neuraminidase (segment 6) indicates an 11 aa deletion in the stalk region. Virus indicated by a broken oval represents a hypothetical reassortant. Source viruses for a reassortant virus are adjacent to the arrow tails; arrow heads point to the resulting reassortant viruses. Colored bars indicate poultry source and year of original isolation: red: chicken, 2013; green: duck, 2013; orange: duck, 2014; purple: chicken, 2014.