4. Tkachenko EA, Bershtein AD, Dzagurova TK, Morozov VG, Slonova RA, Ivanov LI, et al. Actual problems of hemorrhagic fever with renal syndrome [in Russian]. Zh Mikrobiol Epidemiol Immunobiol. 2013;1:51–8. PubMed
5. Tkachenko EA, Dzagurova TK, Bernstein AD, Korotina NA, Okulova NM, Mutnikh ES, et al. Hemorrhagic fever with renal syndrome (history, problems and study perspectives) [in Russian]. Epidemiology and Vaccine Prophylaxis. 2016;15:23–34. https://doi.org/10.31631/2073-3046-2016-15-3-23-34
6. Dzagurova TK, Tkachenko EA, Yunicheva YV, Morozov VG, Briukhanov AF, Bashkirtsev VN, et al. Detection, clinical and etiological characteristics of HFRS in the subtropical zone of the Krasnodar region [in Russian]. Zh Mikrobiol Epidemiol Immunobiol. 2008;1:12–6.
7. Klempa B, Tkachenko EA, Dzagurova TK, Yunicheva YV, Morozov VG, Okulova NM, et al. Hemorrhagic fever with renal syndrome caused by 2 lineages of Dobrava hantavirus, Russia. Emerg Infect Dis. 2008;14:617–25. https://doi.org/10.3201/eid1404.071310
8. Kruger DH, Tkachenko EA, Morozov VG, Yunicheva YV, Pilikova OM, Malkin G, et al. Life-threatening Sochi virus infections, Russia. Emerg Infect Dis. 2015;21:2204–8. https://doi.org/10.3201/eid2112.150891
9. Morozov VG, Ishmukhametov AA, Dzagurova TK, Tkachenko EA. Clinical features of hemorrhagic fever with renal syndrome in Russia [in Russian]. Medical Council. 2017;5:156–61. https://doi.org/10.21518/2079-701X-2017-5-156-161
10. Dzagurova TK, Klempa B, Tkachenko EA, Slyusareva GP, Morozov VG, Auste B, et al. Molecular diagnostics of hemorrhagic fever with renal syndrome during a Dobrava virus infection outbreak in the European part of Russia. J Clin Microbiol. 2009;47:4029–36. https://doi.org/10.1128/JCM.01225-09

Address for correspondence: Evgeniy A. Tkachenko, Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products, Russian Academy of Sciences, Scientific Direction, Premises 8, Building 1, Village of Institute of Poliomyelitis, Settlement Moskovskiy, Moscow 108819, Russia; email: evgeniytkach@mail.ru

Laboratory-Confirmed Avian Influenza A(H9N2) Virus Infection, India, 2019

Varsha Potdar, Dilip Hinge, Ashish Satav, Eric F. Simões, Pragya D. Yadav, Mandeep S. Chadha

Author affiliations: National Institute of Virology, Pune, India (V. Potdar, D. Hinge, P.D. Yadav, M.S. Chadha); Mahan Trust Melghat, Amravati, India (A. Satav); University of Colorado School of Medicine, Aurora, Colorado, USA (E.F. Simões)

DOI: https://doi.org/10.3201/eid2512.190636

A 17-month-old boy in India with severe acute respiratory infection was laboratory confirmed to have avian influenza A(H9N2) virus infection. Complete genome analysis of the strain indicated a mixed lineage of G1 and H7N3. The strain also was found to be susceptible to adamantanes and neuraminidase inhibitors.

Low-pathogenicity avian influenza A(H9N2) viruses have a wide host range, and outbreaks in poultry have been recorded since the 1990s in China (*1*). In India, avian specimens indicated no serologic evidence of H5N1 and H9N2 during 1958–1981 (*2*); however, 5%–6% persons with direct exposure to poultry had H9N2 antibodies (*3*). Human cases of influenza H9N2 virus infection have been observed in Hong Kong, China, Bangladesh, and Pakistan (*4*–*7*).

An institutional review board approved an ongoing community-based surveillance in 93 villages of Korku tribes in Melghat District, Maharashtra State, India, to determine incidence of respiratory syncytial virus (RSV)–associated deaths among children <2 years of age. A total of 2,085 nasopharyngeal swabs from children with severe or fatal pneumonia were transported to India's National Institute of Virology to test for influenza, RSV, and other respiratory viruses. A nasopharyngeal swab from a 17-month-old boy received on February 12, 2019, tested positive by PCR for influenza A(H9N2) virus.

The child, a resident of Melghat, had fever, cough, breathlessness, and difficulty feeding for 2 days after illness onset on January 31, 2019. His high intermittent grade fever had no diurnal variation and no association with rash or mucocutaneous lesions. Examination revealed a conscious, restless child with a respiratory rate of 48 breaths/min and lower chest wall in-drawing with intermittent absence of breathing for ≥20 seconds. He was fully immunized for his age, with bacillus Calmette–Guérin, diphtheria, hepatitis B, poliovirus, and measles vaccines. Both length and weight for age were less than –3 SD. History of travel with his parents to a local religious gathering 1 week before symptom onset was elicited. The father had similar symptoms on return from the gathering but could not undergo serologic testing because of his migrant work. No history of poultry exposure was elicited. The child received an antibacterial drug and antipyretics and recovered uneventfully.

We tested the clinical sample using duplex real-time PCR for influenza A/B, H3N2, and 2009 pandemic H1N1 viruses; RSV A/B; human metapneumovirus; parainfluenza virus types 1–4; rhinovirus; and adenovirus. The sample was strongly positive for influenza A virus (cycle threshold value 20) but negative for seasonal influenza viruses and all respiratory viruses. Real-time PCR analysis for avian influenza viruses H5N1, H7N9, H10N8, and H9N2

revealed positivity for H9N2 virus (cycle threshold value for H9 was 25). We confirmed this result by sequencing the matrix (M) and hemagglutinin (HA) genes of the isolate, designated A/India/TCM2581/2019/(H9N2); the M gene (260 bp) had 97.27% nucleotide identity with A/chicken/India/99321/2009(H9N2), and the HA gene (225,478 bp) had 96.93% nucleotide identity with A/chicken/India/12CL3074/2015(H9N2).

We then generated whole-genome sequences by using the Miniseq NGS Platform (Illumina, https://www.illumina.com) and a de novo assembly program (CLC Genomics Software 10.1.1 [*8*]). We used MEGA7 (https://megasoftware.net) with a Tamura-Nei nucleotide substitution model including 1,000 replicates bootstrap support (*9*) for evolutionary analysis of 8 genes of A/India/TCM2581/2019/(H9N2) (submitted to GenBank under accession nos. MK673893–900). The HA, neuraminidase, and nucleoprotein gene phylogeny of A/India/TCM 2581/2019/(H9N2) grouped with the dominant G1 lineage (h94.1.1) and clustered with poultry strains from India and human strains from Bangladesh (Figure). The M, nonstructural, polymerase basic 1, polymerase basic 2, and polymerase acidic genes were related to an H7N3 isolate from Pakistan (*10*) (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/25/12/19-0636-App1.pdf). We confirmed that the A/India/TCM2581/2019(H9N2) strain had low pathogenicity, showing a KSKR/GLF amino acids motif at the cleavage site of HA (335-341 [H9 numbering]). We observed 6 potential glycosylation sites (11, 87, 123, 280, 287, and 472 [H9 numbering]) and loss of 2 sites (208 and 218 [H9 numbering]) in the HA gene of A/India/TCM2581/2019(H9N2) with respect to G1 viruses.

The virus was susceptible to adamantanes with S31 and to neuraminidase inhibitor with R292 and E119 (N2 numbering) (*11*). A/India/TCM2581/2019(H9N2) had Q226L and I155T in HA gene, which promote the human receptor binding. Compared with G1 vaccine strain A/Hong Kong/1073/99, the study strain had multiple mammalian-specific mutations that already exist in poultry-adapted H9N2. The study strain had amino acid changes R207K, H436Y, and M677T in the polymerase basic 1 gene; A515T in the polymerase acidic 1 gene; N30D, T215A, and T139A (all H3 numbering) in the matrix 1 gene; and P42S in the nonstructural 1 gene, all of which are known to be associated with mammalian host specificity and increased virulence in ferrets and mice (*12*). Known markers for virulence and transmission (E627K and D701N) in the polymerase basic 2 gene in the study strain were absent (Appendix Table 1).

Bayesian evolutionary analyses using BEAST version 1.8.1 (*13*) of the HA gene of H9N2 poultry strains from India indicated 3 clusters of multiple introductions at the estimated node age of 2000–2001 (Appendix Figure 2). Human strain A/India/TCM2581/2019(H9N2) and the other poultry viruses from India evolved with 5.163×10^{-3} substitutions/site/year.

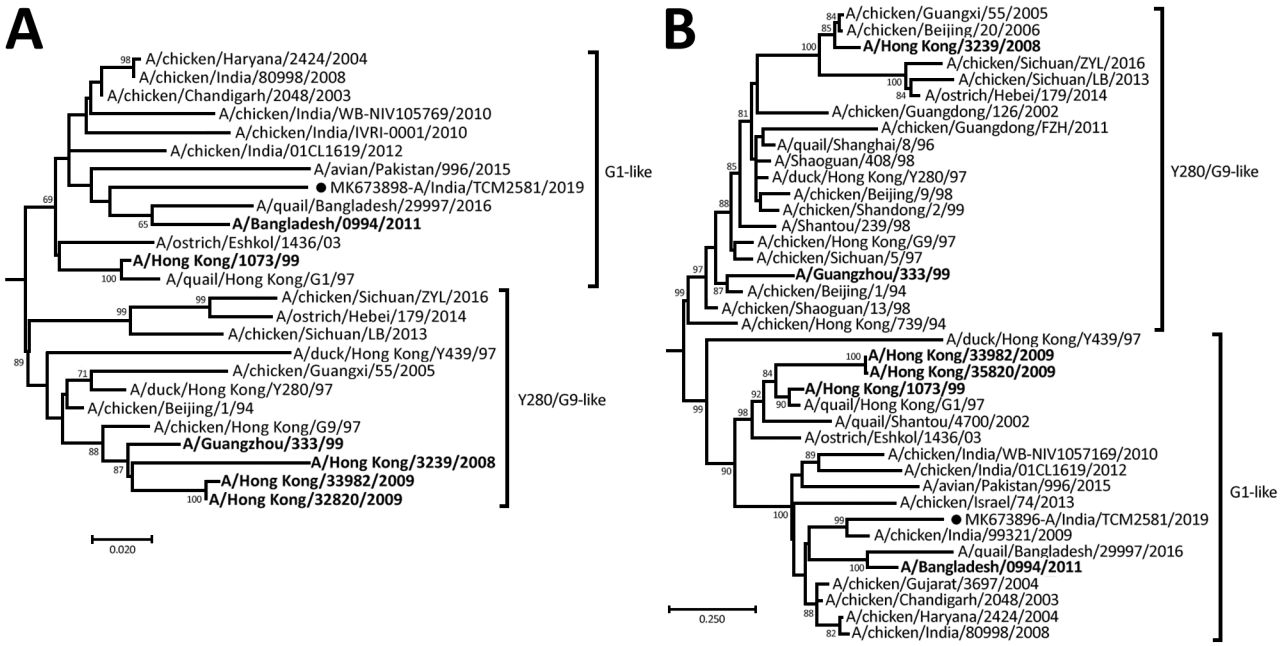


Figure. Phylogenetic tree of hemagglutinin gene (A) and neuraminidase gene (B) gene of influenza virus A/India/TCM 2581/2019(H9N2) from India (black circle) and reference strains. The numbers above the branches are the bootstrap probabilities (%) for each branch, determined by using MEGA 7.0 (https://megasoftware.net). Human cases from other countries are in bold. Scale bars indicate nucleotide substitutions per site.

In conclusion, multiple introductions of H9N2 viruses in poultry have been observed in India. The identification of a human case of H9N2 virus infection highlights the importance of systemic surveillance in humans and animals to monitor this threat to human health.

Acknowledgments

We thank Devendra Mourya for his valuable suggestions and support during the study. We also thank Niteen Wairagkar for critical review of the project. We acknowledge the health workers from Mahan Trust Melghat for their help in collecting clinical information and sample collection. We also acknowledge Sumit Bhardwaj and the staff of the Influenza Group and Biosafety Level 4 Laboratory at the National Institute of Virology for scientific and technical support for influenza testing and next-generation sequencing. Finally, we acknowledge the state Integrated Disease Surveillance Programme in Maharashtra for carrying out the preliminary house-to-house investigation.

Financial support was provided by the Bill and Melinda Gates Foundation (grant no. OPP1128488) to E.F.S.

About the Author

Dr. Potdar is senior scientist heading the Influenza Group at the National Institute of Virology, Pune, India. Her primary research interest is molecular characterization and antiviral susceptibility of influenza viruses.

References

1. Malik Peiris JS. Avian influenza viruses in humans. Rev Sci Tech. 2009;28:161–73. https://doi.org/10.20506/rst.28.1.1871
2. Pawar SD, Jamgaonkar AV, Umarani UB, Kode SS. Seroepidemiology of avian influenza H5N1, H9N2 and Newcastle disease viruses during 1954 to 1981 in India. Indian J Med Res. 2016;144:472–6. https://doi.org/ 10.4103/0971-5916.198666
3. Pawar SD, Tandale BV, Raut CG, Parkhi SS, Barde TD, Gurav YK, et al. Avian influenza H9N2 seroprevalence among poultry workers in Pune, India, 2010. PLoS One. 2012;7:e36374. https://doi.org/10.1371/journal.pone.0036374
4. Pan Y, Cui S, Sun Y, Zhang X, Ma C, Shi W, et al. Human infection with H9N2 avian influenza in northern China. Clin Microbiol Infect. 2018;24:321–3. https://doi.org/10.1016/j.cmi.2017.10.026
5. Butt KM, Smith GJ, Chen H, Zhang LJ, Leung YH, Xu KM, et al. Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. J Clin Microbiol. 2005;43:5760–7. https://doi.org/10.1128/JCM.43.11.5760-5767.2005
6. Shanmuganatham K, Feeroz MM, Jones-Engel L, Smith GJ, Fourment M, Walker D, et al. Antigenic and molecular characterization of avian influenza A(H9N2) viruses, Bangladesh. Emerg Infect Dis. 2013;19. https://doi.org/10.3201/eid1909.130336
7. Ali M, Yaqub T, Mukhtar N, Imran M, Ghafoor A, Shahid MF, et al. Avian influenza A(H9N2) virus in poultry worker, Pakistan, 2015. Emerg Infect Dis. 2019;25:136–9. https://doi.org/10.3201/eid2501.180618
8. Mourya DT, Yadav PD, Nyayanit DA, Majumdar TD, Jain S, Sarkale P, et al. Characterization of a strain of quaranfil virus isolated from soft ticks in India. Is quaranfil virus an unrecognized cause of disease in human and animals? Heliyon. 2019;5:e01368. https://doi.org/10.1016/j.heliyon.2019.e01368
9. Iqbal M, Yaqub T, Reddy K, McCauley JW. Novel genotypes of H9N2 influenza A viruses isolated from poultry in Pakistan containing NS genes similar to highly pathogenic H7N3 and H5N1 viruses. PLoS One. 2009;4:e5788. https://doi.org/10.1371/journal.pone.0005788
10. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4. https://doi.org/ 10.1093/molbev/msw054
11. Govorkova EA, Baranovich T, Seiler P, Armstrong J, Burnham A, Guan Y, et al. Antiviral resistance among highly pathogenic influenza A (H5N1) viruses isolated worldwide in 2002–2012 shows need for continued monitoring. Antiviral Res. 2013;98:297–304. https://doi.org/10.1016/j.antiviral.2013.02.013
12. Li X, Shi J, Guo J, Deng G, Zhang Q, Wang J, et al. Genetics, receptor binding property, and transmissibility in mammals of naturally isolated H9N2 avian influenza viruses. PLoS Pathog. 2014;10:e1004508. https://doi.org/ 10.1371/journal.ppat.1004508
13. Potdar VA, Hinge DD, Dakhave MR, Manchanda A, Jadhav N, Kulkarni PB, et al. Molecular detection and characterization of influenza ‘C’ viruses from western India. Infect Genet Evol. 2017;54:466–77. https://doi.org/ 10.1016/j.meegid.2017.08.005

Address for correspondence: Varsha Potdar, National Institute of Virology, Ministry of Health and Family Welfare, 20-A Dr. Ambedkar Rd, Post Box 11, Pune, Maharashtra 411001, India; email: potdarvarsha9@gmail.com

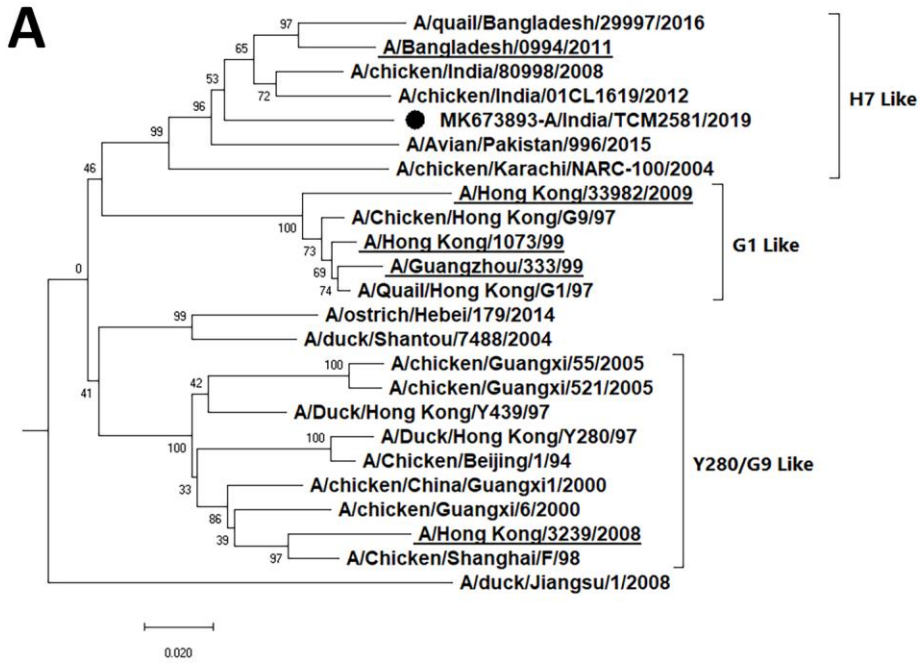
Article DOI: https://doi.org/10.3201/eid2512.190636

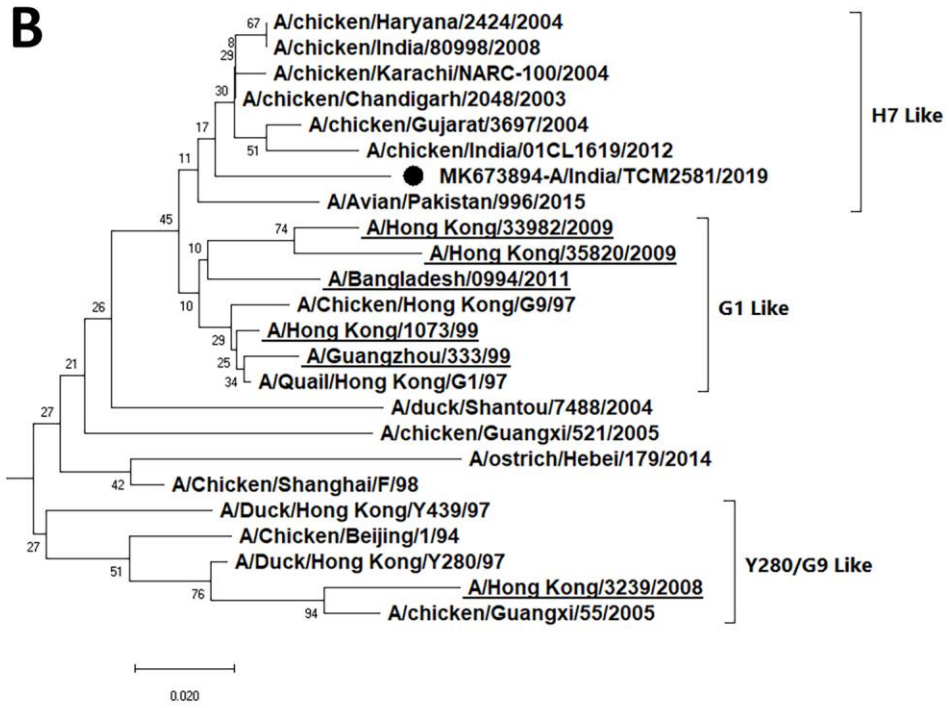
Laboratory-Confirmed Avian Influenza A(H9N2) Virus, India, 2019

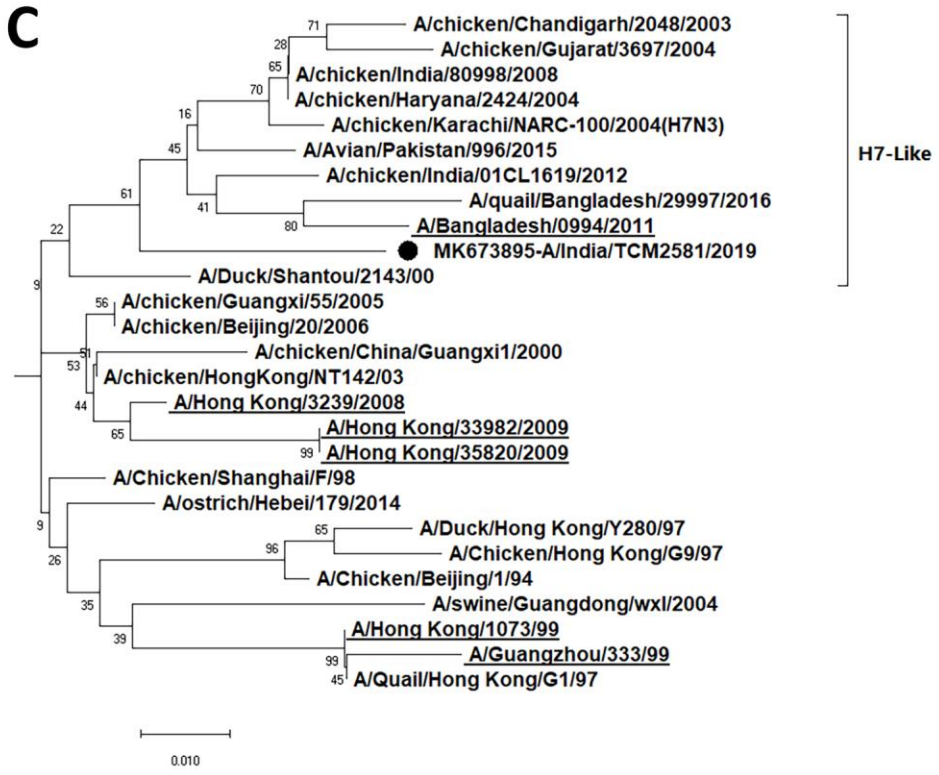
Appendix

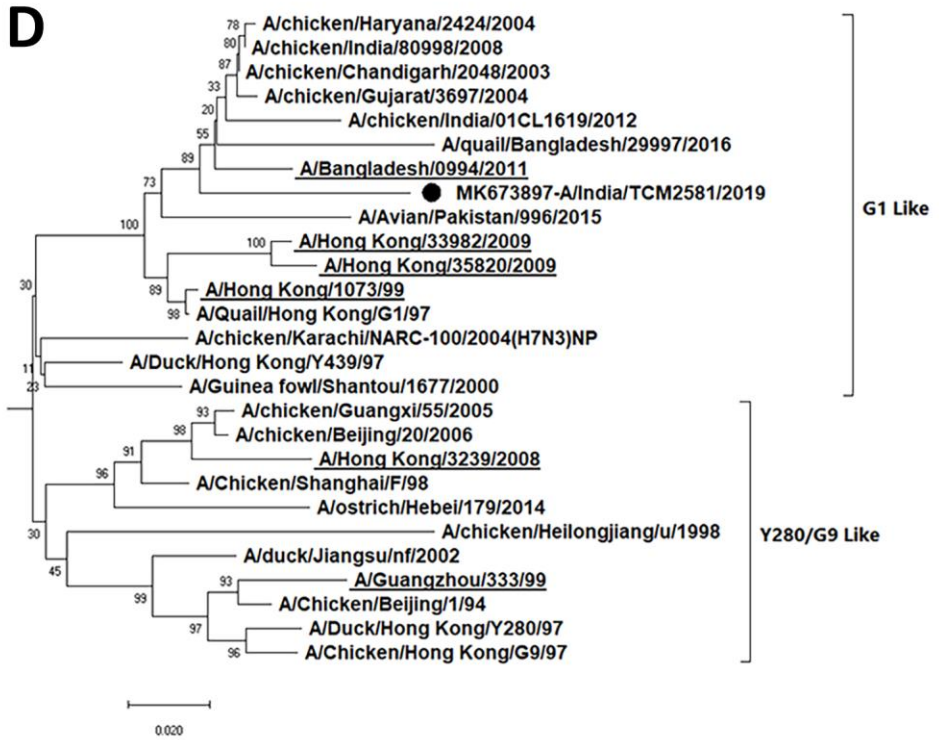
Appendix Table. The study virus showing multiple mammalian specific mutations in all eight genes reported earlier

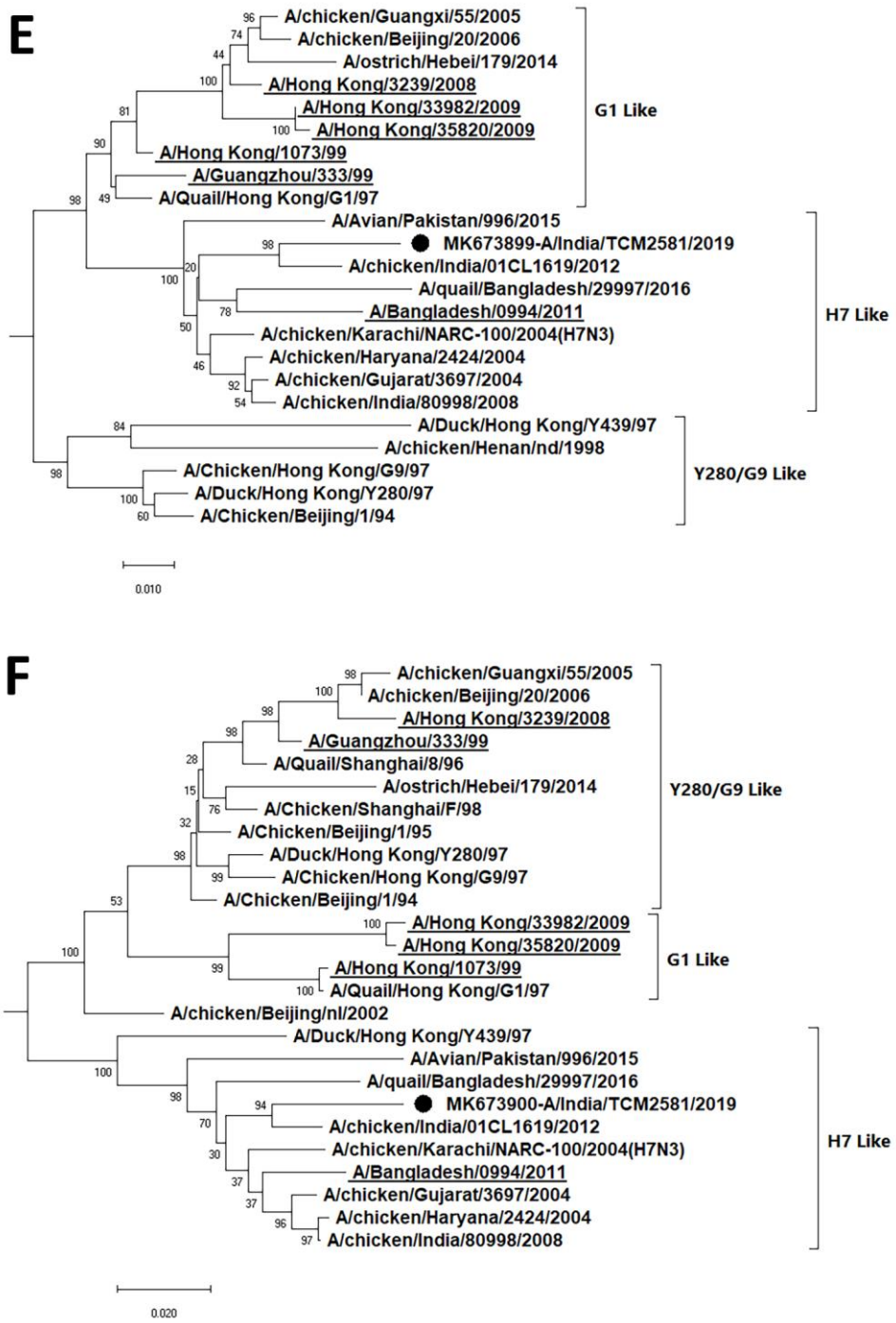
Gene	Substitution	Function	A/India/TCM2581/2019
PB2	E627K	Virulence and transmission ofH5N1 in mammals; enhanced polymerase activity; mammalian host adaptation	E627
	D701N, K147T, M147L, K339T, A588T/I	Polymerase activity in mammalian cells of avian H5N1 mammalian cells; polymerase activity; and virulence by regulating the cap binding activity	D701, M147, K339, A558V
PB1	R207K, H436Y, M677T	Polymerase activity in mammalian cells; polymerase activity and virulence in mallards, ferrets and mice; virulence-related mutation	R207K, H436Y, M677T
PA	A515T	Polymerase activity in mammalian cells	A515T
HA (H3 numbering)	Q226L, I155T, H183N, A190V	Q226L promote the affinity of avian influenza viruses for human type receptors	Q226L, I155T, H183, A190
	HA1/HA2 cleavage site	Polybasic amino acid indicates high pathogenicity	KSKR/GLF
NA (N2 numbering)	49–68 deletion (A/Gs/Gud1/1996)	Enhance virulence in mouse	No deletion
	V116A, E119A/G/V, I221M/V/L/K/R, R292K, H274Y	Reduced susceptibility to zanamivir, oseltamivir and/or peramivir	V116, E119, I221, R292, H274
M1	N30D, T139A, T215A	Mammalian host specific markers, virulence related	N30D, T139N, T215A
	V15I	Mammalian host specific markers; common substitution in H5N1 exhibiting high virulence in mice	V15I
M2	L55F	Mammalian host specific markers, virulence related	L55
	S31N/G	Amantadine resistance	S31
NS1	P42S, F103L, M106I, PL motif	Increased virulence in mice; virulence related “(ESEV, EPEV, or KSEV)”	P42S, F103, M106, KPEV


A
A/quail/Bangladesh/29997/2016
A/Bangladesh/0994/2011
A/chicken/India/80998/2008
A/chicken/India/01CL1619/2012
MK673893-A/India/TCM2581/2019
A/Avian/Pakistan/996/2015
A/chicken/Karachi/NARC-100/2004
H7 Like
A/Hong Kong/33982/2009
A/Chicken/Hong Kong/G9/97
A/Hong Kong/1073/99
A/Guangzhou/333/99
A/Quail/Hong Kong/G1/97
G1 Like
A/ostrich/Hebei/179/2014
A/duck/Shantou/7488/2004
A/chicken/Guangxi/55/2005
A/chicken/Guangxi/521/2005
A/Duck/Hong Kong/Y439/97
A/Duck/Hong Kong/Y280/97
A/Chicken/Beijing/1/94
A/chicken/China/Guangxi1/2000
A/chicken/Guangxi/6/2000
A/Hong Kong/3239/2008
A/Chicken/Shanghai/F/98
Y280/G9 Like
A/duck/Jiangsu/1/2008
0.020

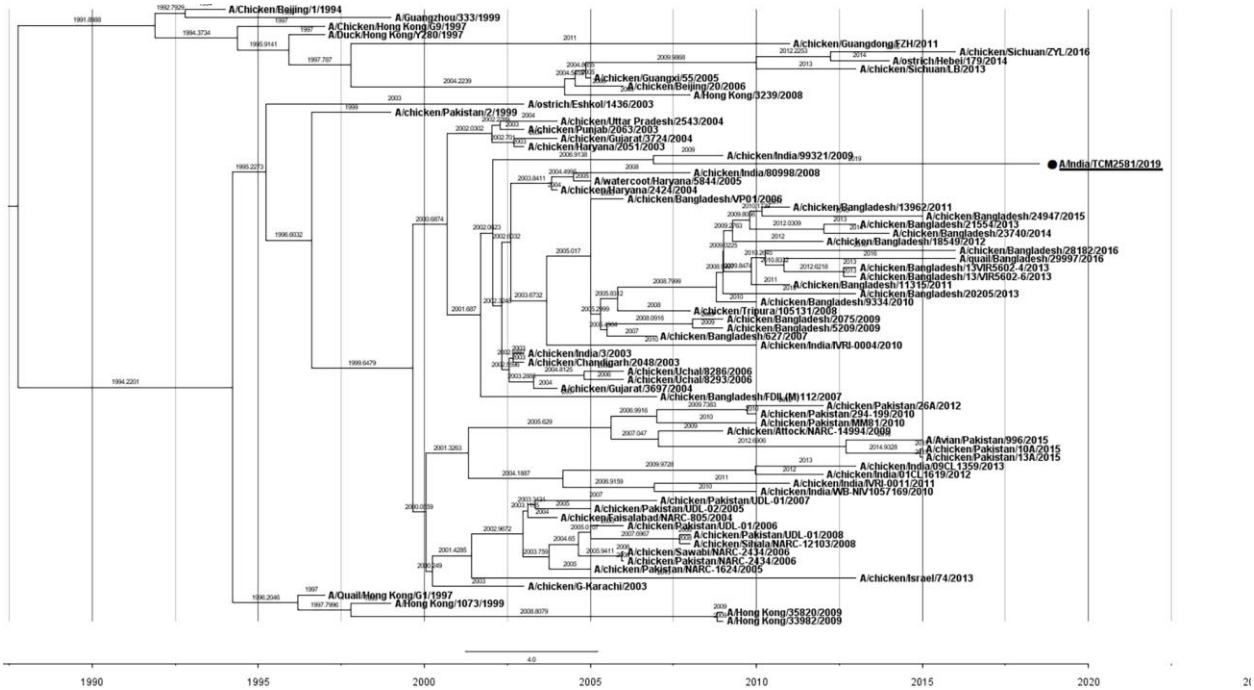

B
A/chicken/Haryana/2424/2004
A/chicken/India/80998/2008
A/chicken/Karachi/NARC-100/2004
A/chicken/Chandigarh/2048/2003
A/chicken/Gujarat/3697/2004
A/chicken/India/01CL1619/2012
MK673894-A/India/TCM2581/2019
A/Avian/Pakistan/996/2015
H7 Like
A/Hong Kong/33982/2009
A/Hong Kong/35820/2009
A/Bangladesh/0994/2011
A/Chicken/Hong Kong/G9/97
A/Hong Kong/1073/99
A/Guangzhou/333/99
A/Quail/Hong Kong/G1/97
G1 Like
A/duck/Shantou/7488/2004
A/chicken/Guangxi/521/2005
A/ostrich/Hebei/179/2014
A/Chicken/Shanghai/F/98
A/Duck/Hong Kong/Y439/97
A/Chicken/Beijing/1/94
A/Duck/Hong Kong/Y280/97
A/Hong Kong/3239/2008
A/chicken/Guangxi/55/2005
Y280/G9 Like
0.020


C
A/chicken/Chandigarh/2048/2003
A/chicken/Gujarat/3697/2004
A/chicken/India/80998/2008
A/chicken/Haryana/2424/2004
A/chicken/Karachi/NARC-100/2004(H7N3)
A/Avian/Pakistan/996/2015
A/chicken/India/01CL1619/2012
A/quail/Bangladesh/29997/2016
A/Bangladesh/0994/2011
MK673895-A/India/TCM2581/2019
A/Duck/Shantou/2143/00
A/chicken/Guangxi/55/2005
A/chicken/Beijing/20/2006
A/chicken/China/Guangxi1/2000
A/chicken/HongKong/NT142/03
A/Hong Kong/3239/2008
A/Hong Kong/33982/2009
A/Hong Kong/35820/2009
A/Chicken/Shanghai/F/98
A/ostrich/Hebei/179/2014
A/Duck/Hong Kong/Y280/97
A/Chicken/Hong Kong/G9/97
A/Chicken/Beijing/1/94
A/swine/Guangdong/wxl/2004
A/Hong Kong/1073/99
A/Guangzhou/333/99
A/Quail/Hong Kong/G1/97
H7-Like
0.010


D
A/chicken/Haryana/2424/2004
A/chicken/India/80998/2008
A/chicken/Chandigarh/2048/2003
A/chicken/Gujarat/3697/2004
A/chicken/India/01CL1619/2012
A/quail/Bangladesh/29997/2016
A/Bangladesh/0994/2011
MK673897-A/India/TCM2581/2019
A/Avian/Pakistan/996/2015
A/Hong Kong/33982/2009
A/Hong Kong/35820/2009
A/Hong Kong/1073/99
A/Quail/Hong Kong/G1/97
A/chicken/Karachi/NARC-100/2004(H7N3)NP
A/Duck/Hong Kong/Y439/97
A/Guinea fowl/Shantou/1677/2000
A/chicken/Guangxi/55/2005
A/chicken/Beijing/20/2006
A/Hong Kong/3239/2008
A/Chicken/Shanghai/F/98
A/ostrich/Hebei/179/2014
A/chicken/Heilongjiang/u/1998
A/duck/Jiangsu/nf/2002
A/Guangzhou/333/99
A/Chicken/Beijing/1/94
A/Duck/Hong Kong/Y280/97
A/Chicken/Hong Kong/G9/97
G1 Like
Y280/G9 Like
0.020



Appendix Figure 1. Phylogenetic tree of six internal gens of study virus A/India/TCM 2581/2019 (H9N2). A) Polymerase basic 2 gene (PB2). B) Polymerase basic 1 gene (PB1). C) Polymerase gene(PA). D) Nuclear Protein gene (NP). E) Matrix gene (M). F) Non structure gene (NS). The numbers above the branches are the bootstrap probabilities (%) for each branch, determined using the MEGA software (version 7). The studied strain is marked with a black dot and the human cases from other countries were underlined.



Appendix Figure 2. Bayesian evolutionary tree of Influenza A(H9N2) virus based on the nucleotide sequence of the HA gene. The study virus A/India/TCM 2581/2019 (H9N2) is underlined and marked with a black dot. The evolutionary years are noted for the node. This Bayesian tree was generated using the BEAST software.