

1 **Notorious Novel Avian Influenza Viruses H10N8 and H7N9 in China**
2 **in 2013 Co-originated from H9N2**

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17 **Running title**

18 Novel AIVs H10N8 and H7N9 co-originated from H9N2

19 **Key words**

20 Avian Influenza Virus; H10N8; H7N9; China

21 **Abstract (179 words)**

22 In 2013, two new avian influenza viruses (AIVs) H7N9 and H10N8 emerged in China
23 caused worldwide concerns. Previous studies have studied their originations
24 independently; this study is the first time to investigate their co-originating
25 characteristics. Gene segments of assorted subtype influenza A viruses, as well as

26 H10N8 and H7N9, were collected from public database. With the help of series
27 software, small and large-scale phylogenetic trees, mean evolutionary rates, and
28 divergence years were obtained successionaly. The results demonstrated the two
29 AIVs co-originated from H9N2, and shared a spectrum of mutations in common on
30 many key sites related to pathogenic, tropism and epidemiological characteristics. For
31 a long time, H9N2 viruses had been circulated in eastern and southern China; poultry
32 was the stable and lasting maintenance reservoir. High carrying rate of AIVs H9N2 in
33 poultry had an extremely high risk of co-infections with other influenza viruses,
34 which increased the risk of virus reassortment. It implied that novel AIVs reassortants
35 based on H9N2 might appear and prevail at any time in China; therefore, surveillance
36 of H9N2 AIVs should be given a high priority.

37 **Introduction**

38 In 2013, two episodes of influenza emerged in China caused worldwide concerns. A
39 new avian influenza virus (AIV) H7N9 subtype first appeared in China on February
40 19, 2013. As of August 31, 2013, the virus had spread to ten provinces (Anhui, Fujian,
41 Guangdong, Hebei, Henan, Hunan, Jiangsu, Jiangxi, Shandong, and Zhejiang) and
42 two metropolitan cities (Shanghai and Beijing). Of 134 patients with the influenza, 45
43 died with a fatality rate of 33.58%, which is much higher compared with the fatality
44 rate (<0.25%) for pandemic influenza H1N1 in 2009-2010 (Li Q et al. 2014; Chen Y
45 et al. 2013). There were no new cases reported during the period from August 31 to
46 October 14, 2013. On October 15, 2013, a severe human case reemerged in Shaoxin
47 City, Zhejiang Province, and as of April 11, 2014, there reported 264 severe human
48 cases (including 4 Cantonese confirmed in Hong Kong, one Cantonese confirmed in
49 Malaysia, and a Hong Kong girl who had Guangdong travel history), of which there
50 were 32 deaths (<http://platform.gisaid.org/epi3/frontend#f7fab>, Access date: April 11,
51 2014; <http://news.sina.com.cn/c/2014-01-28/075929365580.shtml>, Access date:
52 February 7, 2014).

53 On November 30, 2013, a woman aged 73 years presented with fever and was

54 admitted to The First Hospital of Nanchang in Jiangxi Province, China. She
55 developed multiple organ failures and died 9 days after the onset of the disease. A
56 novel reassortant avian influenza A H10N8 virus was isolated from the tracheal
57 aspirate specimen obtained from the patient 7 days after the onset of illness. It is the
58 world's first human case infected by H10N8 subtype A influenza virus (Chen H et al.
59 2014). On February, 2014, another dead case caused by H10N8 avian influenza was
60 reported in Jiangxi Province
61 (<http://www.morningpost.com.cn/xwzx/guonei/2014-02-14/550791.shtml>, Access
62 date: February 14, 2014).

63 Sequence analyses of genomes revealed that these viruses were of avian origin,
64 with six internal segments from avian influenza A H9N2 viruses (Chen H et al. 2014;
65 Hu Y et al. 2013). The results done by the basic local alignment search tool (BLAST,
66 <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) showed most homogenous sequences of the six
67 internal segments associated with Asian strains isolated in between 2005 and 2013. In
68 this study, we analyzed the phylogenetic relationship between the two novel avian
69 influenza viruses H10N8 and H7N9, and explore their commonality in origination and
70 evolution.

71 **Results**

72 **Data collecting**

73 A total of 4593 PB2, 4535 PB1, 4785 PA, 4688 NP, 5379 MP, and NS 5410 sequences
74 were obtained. They all affiliated to isolates established in Asia during the period
75 from January 1, 2004 to December 31, 2013. After convergence pre-treatment by
76 means of MEGA6, the matrixes used for large-scale phylogenetic analysis
77 successively contained 827 PB2, 813 PB1, 827 PA, 870 NP, 908 MP, and 890 NS.

78 Six additional small-scale matrixes of China novel AIVs H10N8 and H7N9
79 contained 77 PB2, 73 PB1, 75 PA, 79 NP, 80 MP, and 80 NS gene sequences
80 respectively.

81 **Large-scale/small-scale phylogenetic trees of six interior segments**

82 According to jModeltest, nucleotide substitution models for best maximum likelihood
83 (ML) tree were as follows:

- 84 PB2, GTR+G (AIC=14533·1749),
- 85 PB1, GTR+I+G (AIC=16791·8743),
- 86 PA, GTR+G (AIC=32747·0324),
- 87 NP, GTR+I+G (AIC=13637·1612),
- 88 MP, GTR+I+G (AIC=7529·0599),
- 89 NS, GTR+I+G (AIC=7215·4836).

90 Large-scale ML phylogenetic trees were constructed according to the
91 abovementioned substitution models by MEGA6. The results showed that influenza A
92 viruses had a remarkable tendency of clustering according to their subtype and
93 geographical distribution. Novel AIVs H10N8 and H7N9 shared very close
94 homogeneity, and might originated from China AIV H9N2 circulated for long time in
95 eastern China such as Shanghai and the provinces of Zhejiang, Jiangsu, Anhui, and
96 Shandong.

97 On PB2, the donors H9N2 had emerged early in 2009 in Shanghai
98 (KC768062DK; KC779062SW, DK or SW after the number was the host species, the
99 same as below, DK/duck, SW/swine, CK/chicken). By the end of 2013, their
100 analogues were broadly distributed in Jiangsu, Zhejiang, Shandong, Hunan, Guangxi,
101 and Hebei. Moreover, in 2013, this segment was sporadic reassorted into an AIV
102 H5N2 in Jiangsu (KF150631CK). The phylogenetic tree also demonstrated that H7N9
103 could at least be divided into two sub-lineages, main lineage and Guangdong lineage.

104 On PB1, the donors H9N2 had circulated in Zhejiang, Shanghai, Jiangsu,
105 Shandong, Henan, Hebei, Beijing, Hunan, Guangdong, and Guangxi since 2009.

106 On PA, the donor H9N2 was isolated early in 2009 in Guangxi (KF367738CK).
107 Since then, its analogous H9N2 had sustained in Shanghai, Zhejiang, Shandong,
108 Hebei, Hunan, Gansu, and Guangxi. Furthermore, this segment even showed a
109 spillover to AIV H6N8 in Guangxi in 2009 (JX304767DK), and H5N2 in Hebei in
110 2010 (JQ041396CK).

111 On MP, the donors H9N2 were detected in eastern China in early 2010

112 (JN869533CK, Jiangsu; JF906209DK, Anhui). During the period from 2010 to 2013,
113 they maintained a continuous epidemic by means of H9N2 in Shanghai, Zhejiang,
114 Jiangsu, Hunan, Guangdong, and also occurred in form of H5N2 in Hebei in 2010
115 (JQ041412CK), so did in form of H5N1 in Hunan in 2011 (CY146711DK).

116 On NS, the donors H9N2 were isolated early in 2007 from multiple-regions. As
117 of 2013, their analogues distributed in Shanghai, Fujian, Zhejiang, Jiangsu, Shandong,
118 Henan, Hebei, Beijing, Hunan, Hubei, Jiangxi, Guangdong, Guangxi, Gansu, and
119 Tibet, and in 2009, it occurred once in Xinjiang by the form of H5N1 subtype
120 (CY099014Enviroment).

121 On NP, although it seemed that 2013 novel AIVs H10N8 and H7N9 diverged
122 slightly from each other, they could still cluster into the same branch in the
123 phylogenetic tree. As the donors H9N2 for H7N9, several strains were isolated in
124 early 2009 in Shanghai (KC779054SW, KC768049DK, KC768050DK), Guangxi
125 (KF367739CK), and Henan (KC779053SW). Zhejiang, Jiangsu, Shandong, Hunan,
126 Guangdong, and Gansu were also their epidemic areas. The segment was also
127 reassorted into H5N2 in Hebei in 2010 (JQ041404CK). As to H10N8, the donors
128 H9N2 and its analogues were isolated Anhui (JF906207CK) in 2010 and Hunan
129 (KF714784CK, 2011; KF714776CK, 2012) in 2011 and 2012 (Fig. 1 and 2,
130 Supplemental Fig. 1).

131 **Mean rate and tMRCA**

132 Six interior segments of influenza A viruses isolated in Asia on large-scale had their
133 mean evolutionary rate ranged $2.92 \times 10^{-3}/\text{Yr.bp} \sim 4.12 \times 10^{-3}/\text{Yr.bp}$ in 2004 - 2013,
134 among which, PB2 and PB1 had a faster mutation rate compared with NS and NP
135 (Tab. 1).

136 According to the allowable maximum variations and allowable maximum
137 distances, each of the six small-scale ML phylogenetic trees containing merely AIVs
138 H10N9 and H7N9 should be divided into more than 8 clades (Supplemental Fig. 2),
139 and these patterns must not be formed within one year. In other words, sequences in
140 each small-scale matrix of the six interior segments must have common ancestors

141 earlier than 2013. Analysis of tMRCA (the time to the most recent common ancestor)
142 confirmed this inference: the most recent common ancestor of China novel AIVs
143 H10N8 and H7N9 all emerged before March 2012, and many segments, such as PB2,
144 might had reassorted in H9N2 in early 2011, and then into these two novel AIVs (Tab
145 1, Fig. 3).

146 **Deduced amino acid alignments**

147 2013 China novel AIVs H10N8 and H7N9 shared a spectrum of mutations in common
148 on many key sites, e.g., Leu89Val and Glu627Lys on PB2; Ile368Val on PB1;
149 Asn30Asp and Thr215Ala on M1; Ser31Asn on M2; and Pro42Ser on NS1, and so on.
150 Especially in PB2, similar to many human derived H7N9 viruses, H10N8 had a
151 variation of Glu627Lys. This motif, to a certain extent, was thought to be a
152 characteristic for the human tropism of AIVs, and could improve viral replication at
153 33°C. On some other sites, deduced amino acid of H10N8 differed from that of H7N9
154 (Tab. 2).

155 **Discussion**

156 The novel AIVs H10N8 and H7N9 identified in China in 2013 had a common origin.
157 AIV H9N2 circulated everlastingly in eastern China had donated six interior segments
158 for their genomes. Their interior segments had evolutionarily achieved in H9N2 likely
159 in 2011 -2012. Having accomplished two spillovers from poultry to human being by
160 means of H10N8 and H7N9 in such a short period, H9N2 demonstrated its high
161 frequency and efficiency on virus reassortment.

162 The two notorious novel AIVs shared a spectrum of mutations in common on
163 many key sites implied that they might have the similar pathogenic, tropism and
164 epidemiological characteristics. It is important to strengthen the surveillance and to
165 reinforce case management of novel AIV H10N8 and novel AIV H7N9.

166 The first strain of H9N2 AIV was isolated in 1966, and soon a large number of
167 infections caused by H9N2 in poultry were reported (Homme P and Easterday B.

168 1970). In 1988, Perez et al (2003) established three H9N2 AIVs from quail in Hong
169 Kong, this is the first time of confirming H9N2 prevalence in Asia poultry. In
170 mainland China, the emergence of H9N2 AIV was appeared in 1994 (Guo X et al.
171 2003). So far in southern China and Hong Kong, H9N2 are divided into three
172 sub-lineages, A/Chicken/Hong Kong/G9/97(H9N2) (G9-Like),
173 A/Quail/HongKong/G1/97(H9N2) (G1-Like), and
174 A/Duck/HongKong/Y439/97(H9N2) (Y439-Like). Further monitoring showed that
175 two lineages of H9N2 influenza viruses, A/Quail/Hong Kong/G1/97
176 (Qa/HK/G1/97)-like and A/Duck/Hong Kong/Y280/97 (Dk/HK/Y280/97)-like,
177 widely distributed in the live-poultry in southeastern China. More than 16% of cages
178 of quail in the poultry markets contained Qa/HK/G1/97-like viruses, and the
179 Qa/HK/G1/97-like viruses were evolving rapidly, especially in their PB2, HA, NP,
180 and NA genes (Guan Y et al. 2000). In this study, H9N2, as the donor for 2013 China
181 novel AIVs H10N8 and H7N9, is genetically Qa/HK/G1/97-like.

182 H9N2 acting as a donor and providing gene segments to reform a novel subtype
183 influenza virus is a very common phenomenon. Gu et al (2010) isolated one H5N1
184 A/duck/Shandong/009/2008 from an apparently healthy domestic ducks in eastern
185 China in 2008. According to their BLAST results, four interior gene segments (PB2,
186 PB1, PA and M) of this isolate displayed the closest relationship with H9N2 subtype
187 that was prevalent in eastern China, and then this H5N1 was thought as a reassortant
188 virus derived from G1-like H9N2 and H5N1 subtypes. Zhang et al (2009) studied the
189 genetic and antigenic characteristics of H9N2 influenza viruses isolated from poultry
190 in eastern China during the period from 1998 to 2008 and suggested that the
191 Ck/SH/F/98-like H9N2 virus may have been the donor of internal genes of human and
192 poultry H5N1 influenza viruses circulating in Eurasia. Experimental studies showed
193 that some of these H9N2 viruses could be efficiently transmitted by the respiratory
194 tract in chicken flocks. Gene exchanging, i.e. H9N2 derives their segment from other
195 subtype influenza viruses, also was found. Cong et al (2007) reported five swine
196 H9N2 influenza viruses isolated from diseased pigs from different farms possessed
197 H5N1-like sequences of the six interior genes, indicating that they were reassortants

198 of H9 and H5 viruses. Reassortant H9N2 influenza viruses containing H5N1-like PB1
199 genes in southern China were reported by Guan et al (2000) and Dong et al (2011).
200 Such a high frequency of gene exchanging between H9N2 and other subtype
201 influenza viruses implied that novel AIV reassortants based on H9N2 might appear
202 and prevail at any time; therefore, surveillance of H9N2 AIVs, specially, H9N2 AIVs
203 in eastern China, should be given a high priority.

204 Reassortment contributing to the generation of genetic diversity can only occur
205 among viruses, which replicate within same cells. The prerequisite for reassortment is
206 that an individual host simultaneously infected with multiple divergent viral strains
207 and then formed a quasispecies pool consisting of rather closely related members
208 (Padidam M et al. 1999; Robertson D et al. 1995). For a long time, three genetic
209 sub-lineages of H9 virus (G1, G9, and Y439) had been circulated in eastern and
210 southern China, and poultry was the stable and lasting maintenance reservoir (Peiris
211 M et al. 1999; Guo Y et al. 2000). The perennial positive rate of antibody against
212 H9N2, as a typical low pathogenic avian influenza virus and a major contributor to
213 this novel H7N9, fluctuates between 5.3% and 12.8%, and the rate of virus isolation
214 could even reach 9% in poultry, but this did not cause any obvious epidemic with
215 mass poultry deaths (Cheng X et al. 2002; Lin Y et al. 2002). On April 3, 2013, during
216 the epidemic of H7N9, we collected the last samples from live poultry market in
217 Shanghai (on April 4th, Shanghai closed the live bird market); from 300 anal swabs of
218 chicken, goose, duck and pigeon, we detected 7% influenza A virus positive, and all
219 of them were H9N2 subtype. On December 25, 2013, in Jiangsu, another eastern
220 China province, from 268 anal swabs of chicken, goose, duck and pigeon, and fecal
221 samples of wild bird, we obtained 14 H9N2 positives, no other subtype
222 (unpublished). Such a high carrying rate of AIV H9N2 in poultry had an extremely
223 high risk of co-infections with other influenza viruses, which increased the risk of
224 virus reassortment. In fact, the reassortment between H5N1 and H9N2 had frequently
225 occurred in China as abovementioned. Our study hereby provided evidence for the six
226 interior segments had appeared in 2012 or before, and later reassorted into the novel
227 H10N8 and H7N9 AIVs, which caused an epidemic in 2013. So, it is important to

228 strengthen the surveillance and to reinforce case management of novel AIV H10N8
229 and novel AIV H7N9.

230 H9N2 AIVs act not just donors for assembling a novel influenza virus and they
231 could act also as pathogens to contaminate mammals directly. H9N2 is the only
232 one of H9 subtype could function that way (Lin Y et al. 2002; Peiris J et al. 2001). In
233 1998, swine H9N2 virus was isolated in Hong Kong; this is the first time of H9
234 subtype AIV infecting mammalian (Saito T et al. 2001). Peiris et al had investigated
235 live pigs traded from southern China to Hong Kong in 1998-2000 and confirmed that
236 H9N2 had been widely spread among pigs (Peiris J et al. 2001). As for H9N2,
237 adaptation to pig was an important milestone on the way to human infection. In 1998,
238 human H9N2 infections emerged in Shaoguan and Shantou Cities of Guangdong
239 Province (Huang P et al. 2001). Peiris also reported two human cases in Hong Kong
240 in 1999 (Peiris M et al. 1999). Therefore, from the point of view that H9N2 could act
241 as potential human pathogen, there is a need for a strict monitoring of H9N2 AIVs in
242 China.

243 Overall, there is an important public health risk for avian influenza A H7N9. The
244 novel avian influenza A H10N8 might be a new wave in the future. As a typical
245 moderate virulence AIV, H9N2 had a very high carrying rate in poultry; it would
246 inevitably lead to a high frequency of reassortment. We deeply concerned here that
247 the next wave of avian flu in the future is extremely likely to be caused by the
248 reassortants of H9N2, or the variant ones from it. Influenza epidemiological and
249 virological surveillance should be further strengthened, especially on H9N2.

250 **Methods**

251 **Data collecting**

252 Genomes of novel AIV H10N8, named as Jiangxi Donghu, were downloaded from the
253 Global Initiative on Sharing Avian Influenza Data (GISAID) database
254 (<http://platform.gisaid.org/epi3/frontend>) on February 6, 2014, with their GISAID
255 Numbers being EPI_ISL_152846_497477 ~ 497484. The six interior gene sequences

256 of Asian influenza A viruses identified during 2004 to 2013 were collected from the
257 NCBI Influenza Virus Sequence Database
258 (<http://www.ncbi.nlm.nih.gov/genomes/FLU/aboutdatabase.html>), and the search
259 formula “Type+Host+Country/Region+Protein+Subtype+Sequence length+Collection
260 date” was set as “A+any+Aisa+PB1 ~ NS+any+Full-length plus+2004-01-01 ~
261 2013-12-31”. Additional matrixes used for calculating the most recent common
262 ancestor (tMRCA) of H10N8 and H7N9 were searched by the formula
263 “A+any+China+PB1 ~ NS+H7N9+Full-length plus+2013-03-14 ~ 2014-02-07”.
264 In order to facilitate the following analyses, sequences in each matrix were named as
265 “Access No.+Host+Region/Country+Subtype+Year”.

266 MEGA6 (<http://megasoftware.net>) was used for the pre-treatment of genome
267 data. According to the amount of gene sequences, all large-scale matrixes were split
268 into 15 ~ 18 sub-matrixes simultaneously containing H10N8 and representative H7N9
269 strain named A/Zhejiang/DTID-ZJU01/2013(H7N9). After W method alignment, a
270 phylogenetic tree was constructed for each sub-matrix by p-distance. The sequences
271 were kept only one or more for further analysis if 1) they are the sequences with the
272 same HA and NA subtypes, and were isolated from the same area within 2 years, and
273 2) they showed high homogeneity in the phylogenetic tree, since the other might be
274 the same strain but established from different individuals.

275 **Large-scale/small-scale phylogenetic trees of six interior segments**

276 jModeltest 2.1.3 program (<http://darwin.uvigo.es>) was applied to estimate the model’s
277 likelihood value and to search the best ML tree. Three substitution schemes and then
278 24 candidate models were evaluated.

279 After aligned by clustalW using the MEGA6 software, ML trees of six
280 large-scale gene sequence matrixes were constructed, with the nucleotide substitution
281 model selecting conducted by Akaike information criterion (AIC) values obtained
282 from jModeltest 2.1.3. Significance testing was bootstrapped with 1000 replicates. Six
283 small-scale ML phylogenetic trees only containing the corresponding segments
284 affiliated to 2013 China novel AIVs H10N8 and H7N9 were constructed by

285 nucleotide substitution model GTR + I + G; The significance testing was bootstrapped
286 with 1000 replicates, too.

287 **Computation of mean evolutionary rate and tMRCA**

288 Both mean evolutionary rate and tMRCA were calculated by using the BEAST v1.6.1
289 (<http://beast.bio.ed.ac.uk>). Mean evolutionary rate was computed based on six
290 large-scale gene sequence matrixes, while the tMRCA between AIVs H10N8 and
291 H7N9 was deservedly deduced based on six small matrixes that only comprised of the
292 segments affiliated to the novel H10N8 and H7N9 identified in 2013. For tMRCA
293 analysis, the collection dates of AIVs H10N8 and H7N9 were calculated by using the
294 following formula:

$$295 \quad [(month-1) \times 30 + date] / 360 + 2013 \quad (1)$$

296 In large-scale analysis, models derived from jModeltest 2.1.3 were used again for
297 choosing nucleotide substitution, and uncorrelated lognormal relaxed clock model
298 was engaged as clock model, while in tMRCA analysis the model of nucleotide
299 substitution and clock were all set as GTR + I + G and strict respectively. Log files
300 were imported into Tracer v1.5 (<http://beast.bio.ed.ac.uk>) to read the needed data.

301 The allowable maximum variations and allowable maximum distances of each
302 segment were deduced by the obtained data based on large-scale matrixes.

303 **Alignments of deduced amino acid**

304 To compare the mutations occurred in some key sites, open reading frames (ORF) of
305 interior segments affiliated to China novel AIVs H10N8 and H7N9 were translated
306 into amino acid, and alignments were done by using the MEGA6.

307 **Data access**

308 All raw and processed data from this study have been derived from 1), Global
309 Initiative on Sharing Avian Influenza Data (GISAID) database
310 (<http://platform.gisaid.org/epi3/frontend>); 2), NCBI Influenza Virus Sequence

311 Database (<http://www.ncbi.nlm.nih.gov/genomes/FLU/aboutdatabase.html>).

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314 H7N9 to NCBI Influenza Virus Sequence Database and the Global Initiative on
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324 **Disclosure declaration**

325 The authors declare they have no competing interests.

326 **Figure legends**

327 **Figure 1.** Large-scale phylogenetic trees of six interior segments affiliated to Asia
328 influenza A viruses. All phylogenetic trees demonstrated the only branch containing
329 AIVs H10N8 or H7N9, because of too large pixels in primary plots; the latters were
330 uploaded as supplemental figure 1. These results demonstrated that there existed very
331 close evolutionary relationship between 2013 China novel AIVs H10N8 and H7N9,
332 and they basically sited within the same branch on phylogenetic trees. Since these
333 branches mainly composed of AIV H9N2 circulated in eastern and southern China
334 without exception, novel AIVs H10N8 and H7N9 in China in 2013 were considered
335 co-originated genetically from H9N2.

336 **Figure 2.** Distribution of the probable donor AIVs H9N2 and their analogues. As the
337 probable donors for six interior segments of novel AIVs H10N8 and H7N9 in China
338 in 2013, AIVs H9N2 and their analogues distributed extensively in eastern and
339 southern China. Shanghai, Zhejiang, Jiangsu, Anhui, Shandong, Hebei, Hunan, and
340 Guangdong were deeply influenced by these AIVs H9N2 and their analogues, in
341 which, Zhejiang and Shanghai were the severest epidemic areas stricken by H7N9,
342 whereas Jiangxi was the epidemic area of H10N8 .

343 **Figure 3.** Probable evolutionary history of AIVs H10N8 and H7N9 in China in 2013.
344 Displayed the probable procedure by this way novel AIVs H10N8 and H7N9 in China
345 in 2013 had evolved themselves. Here just discussed the fact that the novel AIVs
346 H10N8 and H7N9 in China in 2013 derived their six interior segments from H9N2,
347 regardless whether or not these AIVs H9N2 were the same strain.

348 HA and NA segments consulted references 1, 2, and 5. According to the
349 references, HA segments of H10N8 uncertainly derived from two H10N8 isolated in
350 different years.

351 **Supplemental figure 1.** Whole plot large-scale ML phylogenetic trees of six interior
352 segments affiliated to Asia influenza A viruses. Corresponding to Figure 1, novel
353 AIVs H10N8 and H7N9 in China in 2013 co-originated genetically from H9N2
354 circulated in eastern China. Branches pointed by arrow were those ones on which the
355 2013 China novel AIVs H10N8 or/and H7N9 sited.

356 **Supplemental figure 2.** ML phylogenetic trees of six interior segments affiliated to
357 2013 AIVs H10N8 and H7N9. According to the allowable maximum variations and
358 allowable maximum distances, each tree of six interior segments affiliated to 2013
359 AIVs H10N8 and H7N9 should be divided into more than 8 clades.

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Table1. Mean evolutionary rates, tMRCA, and other parameters about six interior gene segments

Segment	CDS length (bp)	Mean rate (10 ⁻³ /Yr.bp)	95% HPD (10 ⁻³ /Yr.bp)	Allowable maximum variation (bp/Yr)	Allowable maximum distance (cM/Yr)	tMRCA*	95% HPD*
PB2	2280	4.12	2.85, 5.40	12.32	0.0054	2011.31	2010.68, 2011.87
PB1	2274	4.01	3.26, 4.74	10.78	0.0047	2011.81	2011.39, 2012.23
PA	2151	3.36	2.54, 4.46	9.59	0.0045	2011.59	2011.06, 2012.09
NP	1497	3.16	2.56, 3.78	5.66	0.0038	2012.28	2011.99, 2012.54
MP	982	3.75	2.85, 4.78	4.69	0.0048	2012.09	2011.62, 2012.51
NS	838	2.92	1.93, 3.96	3.32	0.0040	2011.74	2011.16, 2012.26

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*Note, tMRCA of 2013 AIVs H10N8 and H7N9, and their corresponding 95% HPD values were computed based on the smaller scale matrixes only containing 2013

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H10N8 and H7N9 segments, while the rest parameters were obtained based on large scale matrixes.

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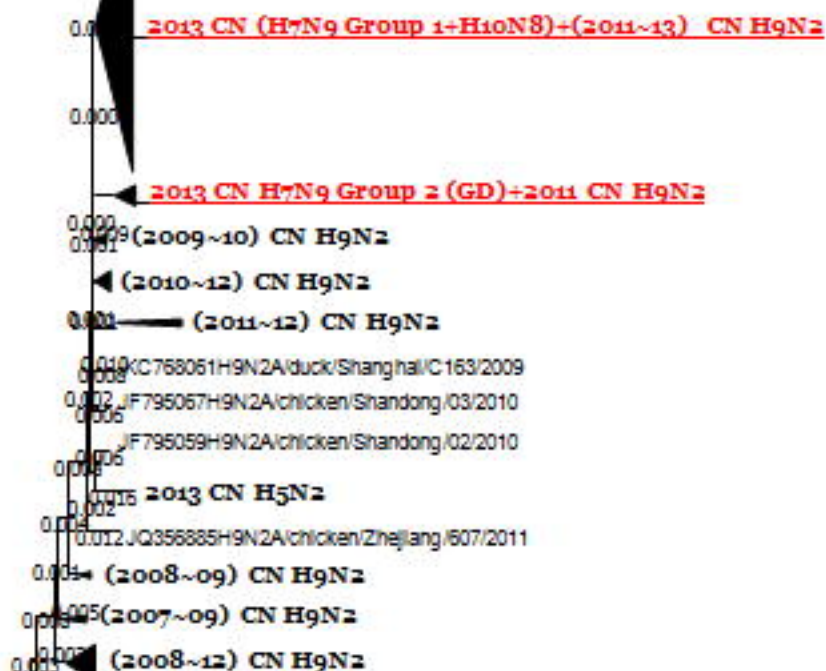
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Table 2. Key mutations in deduced proteins coded by the six interior segments of 2013 AIVs H10N8 and H7N9

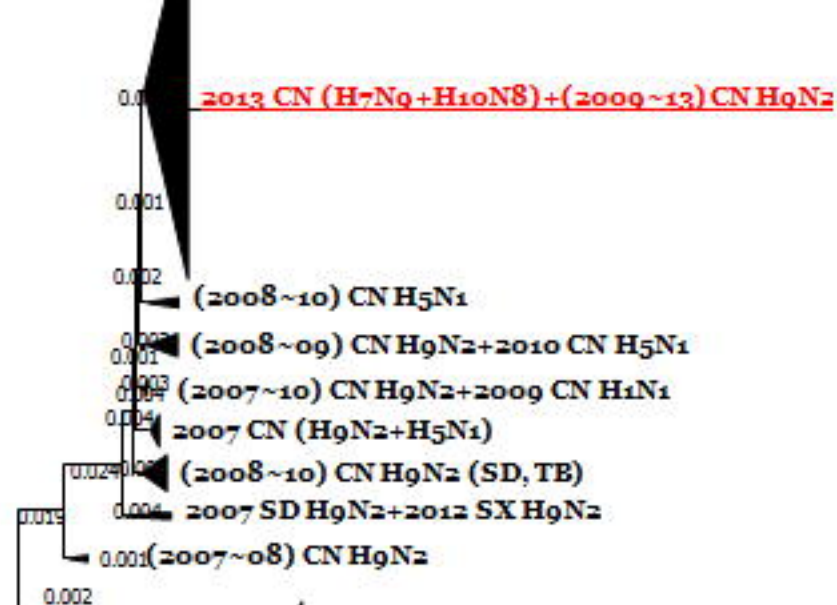
		Known important mutation			Other sites H10N8 differed from H7N9
		H7N9		H10N8	
		Human	AV&EV*		
PB2 (viral replication)					
<i>Leu89Val</i>	Enhanced polymerase activity	29 of 29	46 of 46	1 of 1	<i>Asp87Glu,</i> <i>Asp195Gly,</i> <i>Arg340Lys,</i> <i>Ile411Val,</i> <i>Ala588Val</i>
<i>Glu627Lys</i>	Improved viral replication at 33°C	20 of 29	0 of 46	1 of 1	
<i>Asp701Asn</i>	Mammalian adaptation	2 of 29	0	0	
PB1 (viral replication)					
<i>His99Tyr</i>	Enables droplet transmission	0	0	0	<i>Val14Ala,</i> <i>Lys52Asn,</i> <i>Thr291Ala,</i> <i>Asn642Ser,</i> <i>Lys757Asn</i>
<i>Ile368Val</i>	in ferrets	23 of 26	42 of 45	1 of 1	
PB1-F2 (induce cellular apoptosis and inhibit function of type I interferon)					
Full-length	Needed for virulence in mice	21 of 26	31 of 45	0	
Matrix protein M1 (viral assembly and budding)					
<i>Asn30Asp,</i>	Increased virulence in a mice	31 of 31	47 of 47	1 of 1	0
<i>Thr215Ala</i>	model	31 of 31	47 of 47	1 of 1	
Matrix protein M2					
<i>Ser31Asn</i>	Amantadine resistance	31 of 31	47 of 47	1 of 1	<i>Arg12Lys</i>
NS1 (counteracts host antiviral response)					
<i>Pro42Ser</i>	Increased virulence in mice	31 of 31	47 of 47	1 of 1	<i>Ser206Gly</i>
NP (Nucleoprotein)					
					<i>Ile197Asn,</i> <i>Ile217Asn,</i> <i>Asn482Ser</i>
PA (viral replication)					
					<i>Ala343Ser</i>

* Note: AV=avian; EV=environment

PB2



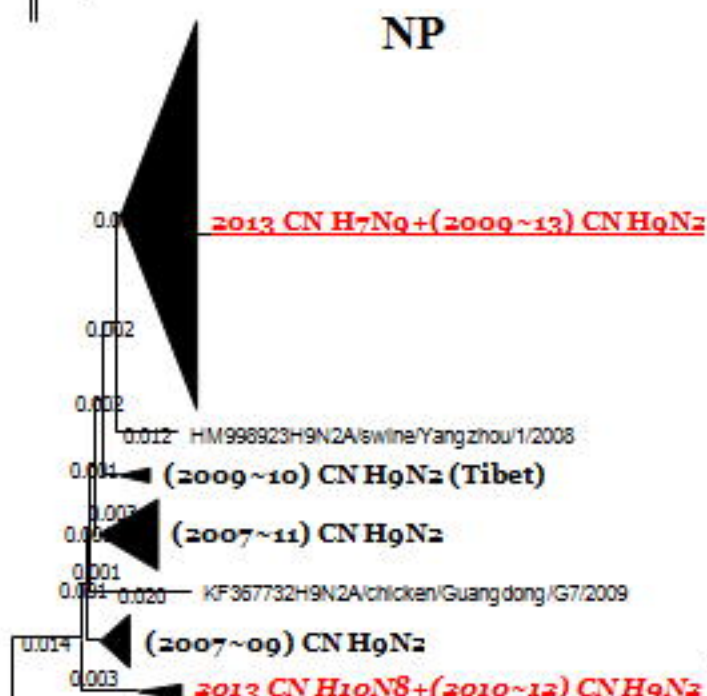
PB1



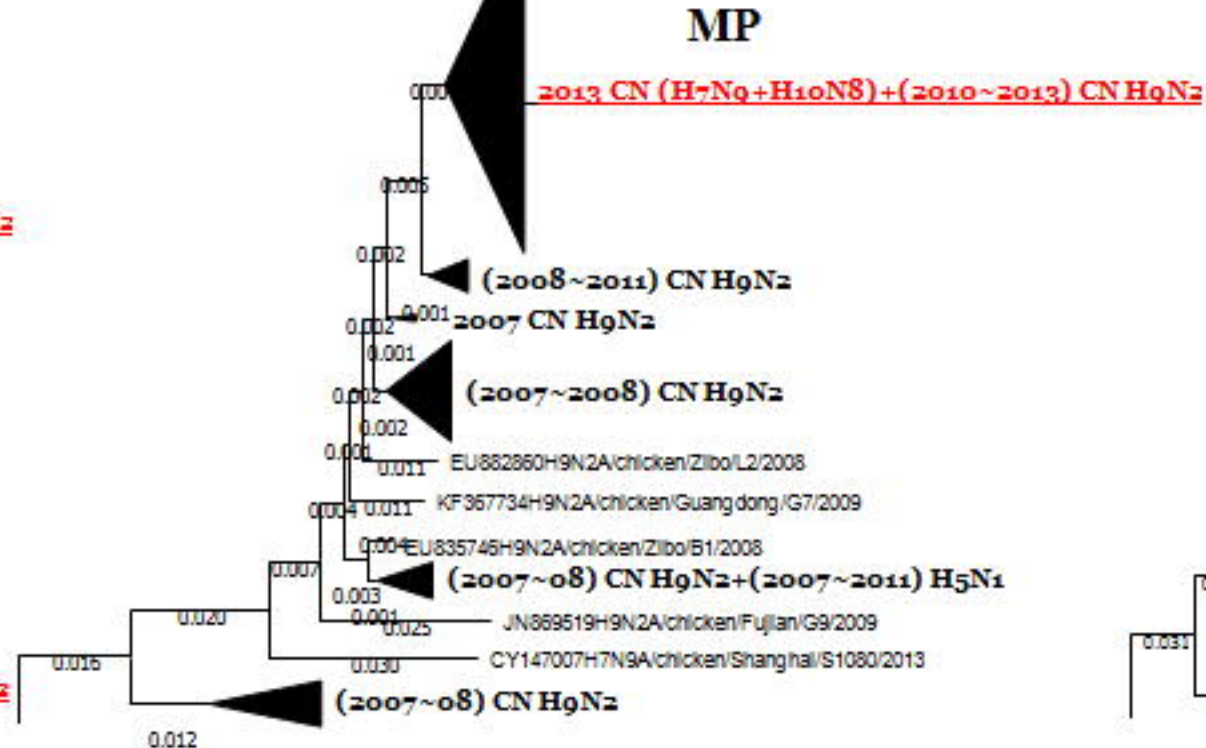
PA



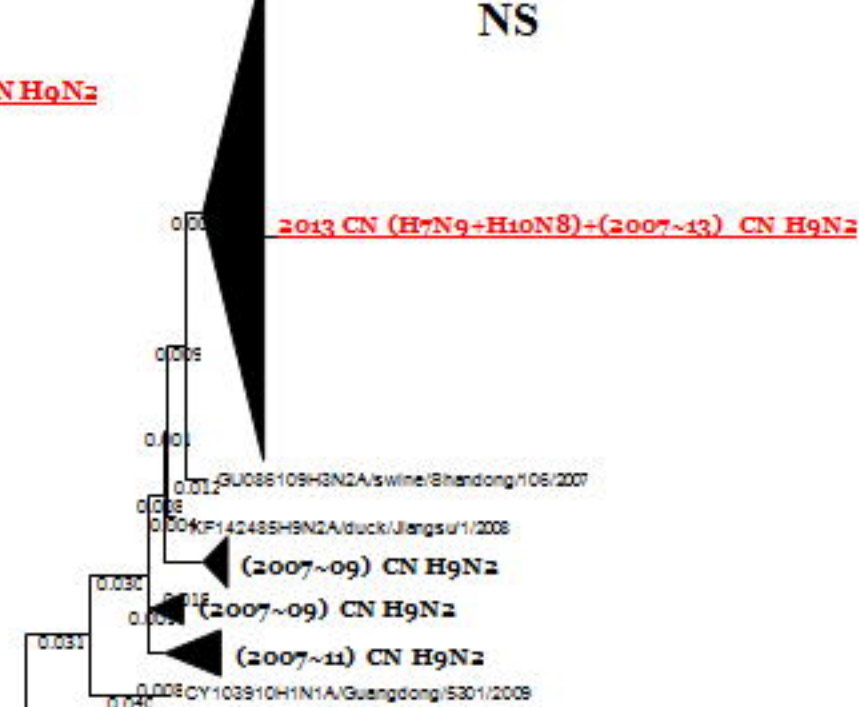
NP

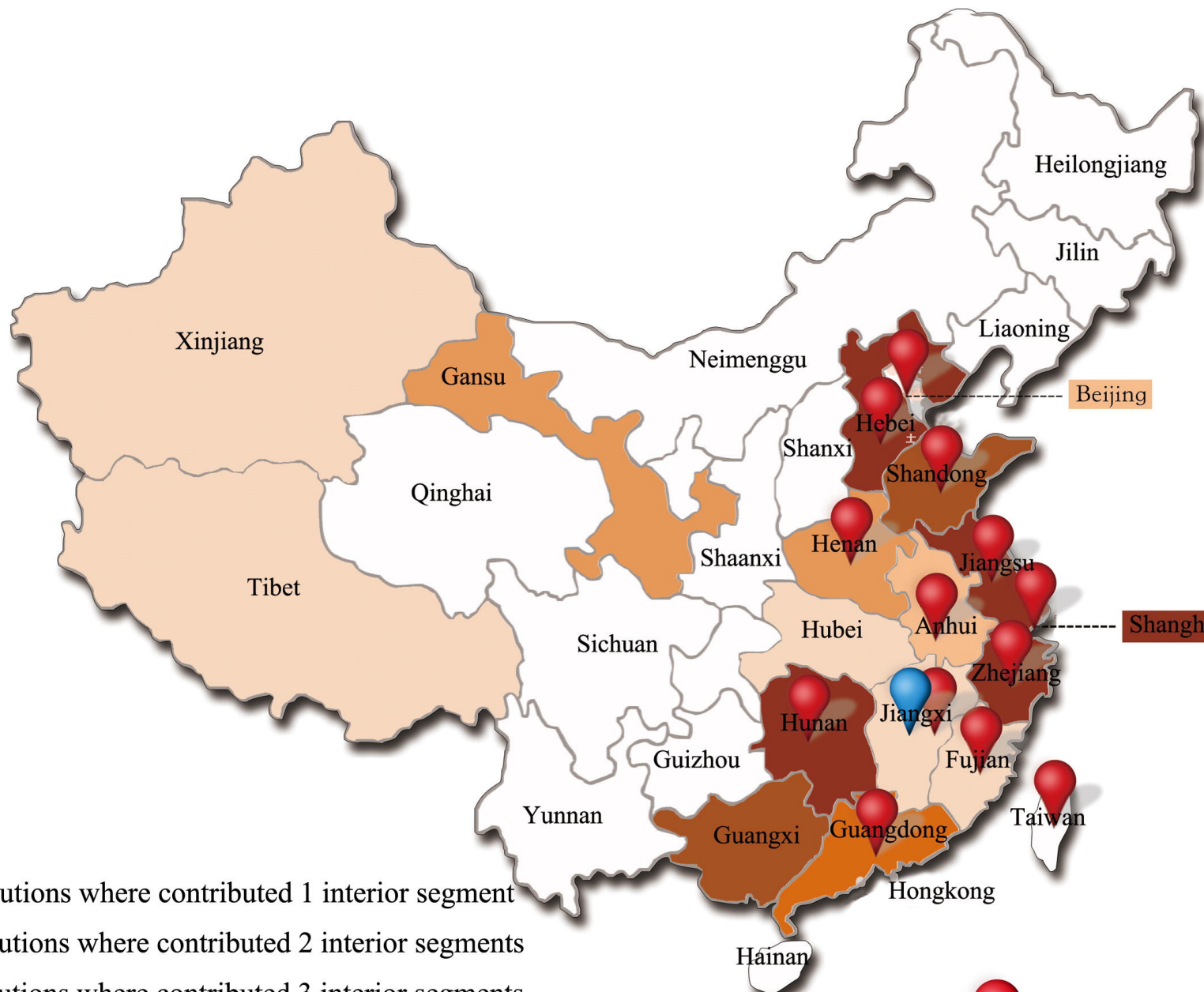


MP



NS





- Distributions where contributed 1 interior segment
- Distributions where contributed 2 interior segments
- Distributions where contributed 3 interior segments
- Distributions where contributed 4 interior segments
- Distributions where contributed 5 interior segments
- Distributions where contributed 6 interior segments

- Distributions of novel AIV H7N9
- Distribution of novel AIV H10N8

