Virology and laboratory diagnosis: Influenza A (H7N9)

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30 March 2013

- Announcement by China NHFPC:
  - 3 cases of human infection with influenza A H7N9
  - 1st Shanghai case:
    - Onset 19 February; passed away 4 March
  - 2nd Shanghai case:
    - Onset 27 February; passed away 10 March
  - Anhui case:
    - Onset 15 March

1 April 2013

- Genetic sequences (all 8 segments of all three virus isolates) already uploaded to Global Initiative on Sharing All Influenza Data (GISAID)
- M2 gene: S31N mutation
- Neuraminidase inhibitor assay: Phenotypically susceptible

PB2	A/Anhui/1/2013 | 1	A/Shanghai/1/2013 | 1	A/Shanghai/2/2013 | 1
A/Anhui/1/2013 | 1	ID	0.996 (2273/2280)	0.999 (2278/2280)
A/Shanghai/1/2013 | 1		ID	0.996 (2271/2280)
A/Shanghai/2/2013 | 1			ID
PB1	A/Anhui/1/2013 | 2	A/Shanghai/1/2013 | 2	A/Shanghai/2/2013 | 2
A/Anhui/1/2013 | 2	ID	0.993 (2260/2274)	0.999 (2272/2274)
A/Shanghai/1/2013 | 2		ID	0.993 (2260/2274)
A/Shanghai/2/2013 | 2			ID
PA	A/Anhui/1/2013 | 3	A/Shanghai/1/2013 | 3	A/Shanghai/2/2013 | 3
A/Anhui/1/2013 | 3	ID	0.998 (2147/2151)	0.999 (2150/2151)
A/Shanghai/1/2013 | 3		ID	0.998 (2148/2151)
A/Shanghai/2/2013 | 3			ID
HA	A/Anhui/1/2013 | 4	A/Shanghai/1/2013 | 4	A/Shanghai/2/2013 | 4
A/Anhui/1/2013 | 4	ID	0.992 (1671/1683)	0.999 (1682/1683)
A/Shanghai/1/2013 | 4		ID	0.992 (1670/1683)
A/Shanghai/2/2013 | 4			ID
NP	A/Anhui/1/2013 | 5	A/Shanghai/1/2013 | 5	A/Shanghai/2/2013 | 5
A/Anhui/1/2013 | 5	ID	0.976 (1462/1497)	1 (1497/1497)
A/Shanghai/1/2013 | 5		ID	0.976 (1462/1697)
A/Shanghai/2/2013 | 5			ID
NA	A/Anhui/1/2013 | 6	A/Shanghai/1/2013 | 6	A/Shanghai/2/2013 | 6
A/Anhui/1/2013 | 6	ID	0.993 (1389/1398)	0.998 (1396/1398)
A/Shanghai/1/2013 | 6		ID	0.993 (1389/1398)
A/Shanghai/2/2013 | 6			ID
M	A/Anhui/1/2013 | 7	A/Shanghai/1/2013 | 7	A/Shanghai/2/2013 | 7
A/Anhui/1/2013 | 7	ID	0.998 (981/982)	1 (982/982)
A/Shanghai/1/2013 | 7		ID	0.998 (981/982)
A/Shanghai/2/2013 | 7			ID
NS	A/Anhui/1/2013 | 8	A/Shanghai/1/2013 | 8	A/Shanghai/2/2013 | 8
A/Anhui/1/2013 | 8	ID	0.997 (836/838)	1 (838/838)
A/Shanghai/1/2013 | 8		ID	0.997 (836/838)
A/Shanghai/2/2013 | 8			ID

Existing laboratory strategy

- Direct detection
  - Antigen: Influenza A
  - Nucleic acid:
    - Influenza A: M gene
    - Seasonal influenza: H1pdm09, H3
    - Non-seasonal influenza: H5, H7, H9
- Viral culture
  - Cell culture (MDCK cell line)

Specific detection tests

- Based on published genetic sequences
  - M gene: Verification of sensitivity
  - H7 gene: Design of specific test against current strain
  - N9 gene: Design of specific test against current strain
- Test optimization
  - Sensitivity and specificity: Real-time PCR
  - Timeliness: One-step protocol with reverse transcription

5 April 2013

- Availability of specific tests for H7 and N9 genes
- Service provision:
  - Testing for notified cases (regardless of fulfilment of reporting criteria)
  - Minimum test set: M, H1pdm09, H3 and H7
  - Within service hours: Two runs per day
  - Outside service hours (based on epidemiological and clinical judgement)

11 April 2013

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Human Infection with a Novel Avian-Origin Influenza A (H7N9) Virus

Rongbao Gao, M.D., Bin Cao, M.D., Yunwen Hu, M.D., Zijian Feng, M.D., M.P.H., Dayan Wang, M.D., Wanfu Hu, M.D., Jian Chen, M.D., Zhijun Jie, M.D., Haibo Qiu, M.D., Ph.D., Ke Xu, M.D., Xuewei Xu, M.D., Hongzhou Lu, M.D., Ph.D., Wenfei Zhu, M.D., Zhancheng Gao, M.D., Nijuan Xiang, M.D., Yinzhong Shen, M.D., Zebao He, M.D., Yong Gu, M.D., Zhiyong Zhang, M.D., Yi Yang, M.D., Ph.D., Xiang Zhao, M.D., Lei Zhou, M.D., Xiaodan Li, M.D., Shumei Zou, M.D., Ye Zhang, M.D., Xiyan Li, M.D., Lei Yang, M.D., Junfeng Guo, M.D., Jie Dong, M.D., Qun Li, M.D., Libo Dong, M.D., Yun Zhu, M.D., Tian Bai, M.D., Shiwen Wang, M.D., Pei Hao, M.D., Weizhong Yang, M.D., Yanping Zhang, M.D., Jun Han, M.D., Hongjie Yu, M.D., Dexin Li, M.D., George F. Gao, Ph.D., Guizhen Wu, M.D., Yu Wang, M.D., Zhenghong Yuan, Ph.D., and Yuelong Shu, Ph.D.

Avian influenza infecting human

- Pathogenicity:
  - High: H5, H7
  - Low: H7, H9, H10
  - Definition:
    - Haemagglutinin containing multiple basic amino acids at cleavage site
    - Correlation with pathogenicity in wild birds/domestic poultry

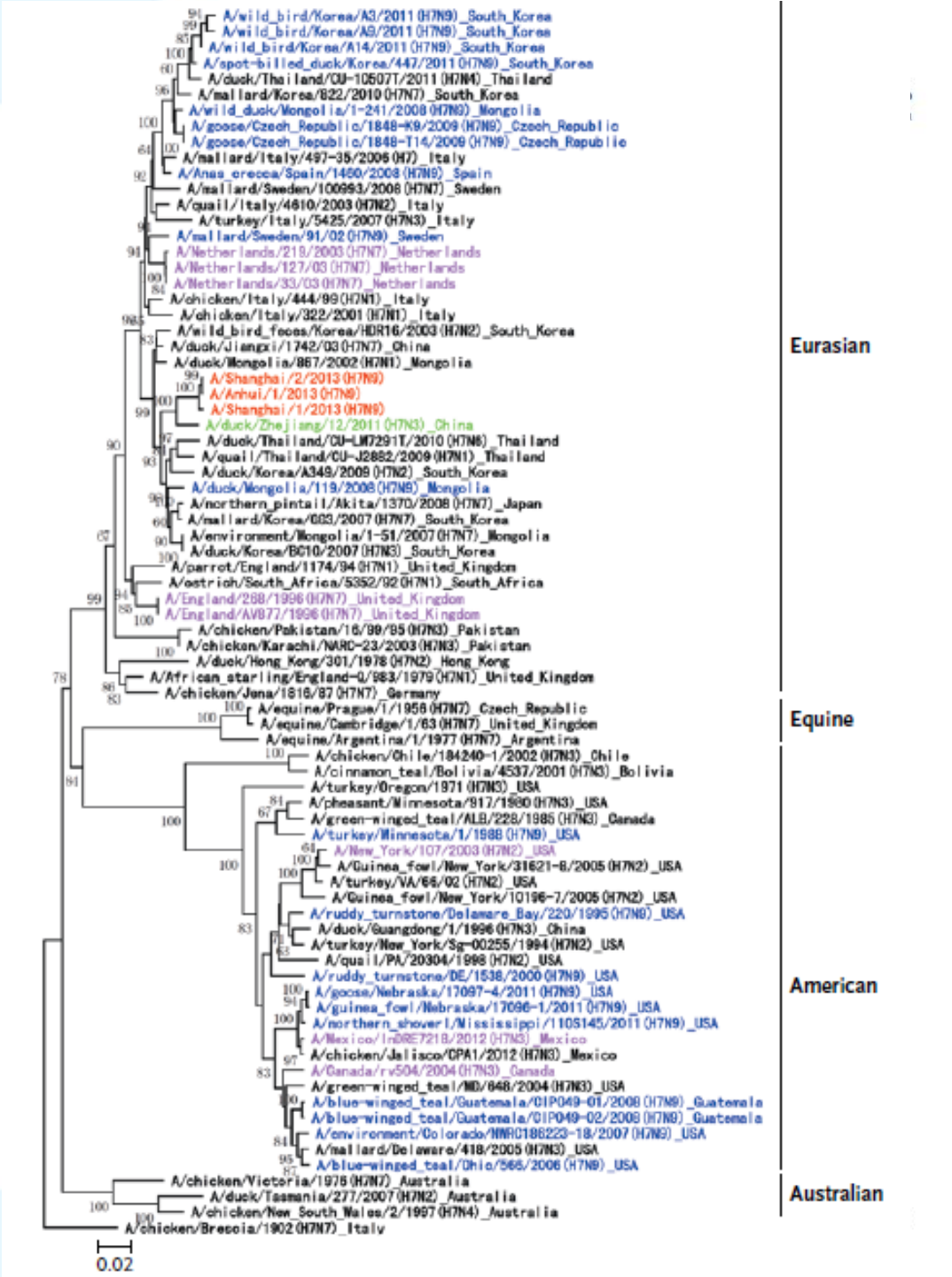
Virology testing

- Throat swab specimens
- Definition of case:
  - Detection of RNA (real-time RT-PCR against respiratory viruses) with subtyping by specific H1-16 and N1-9 specific real-time RT-PCRs
  - Isolation of virus (allantoic and amniotic sac of 9-11-day embryonated hen's egg for 48-72 hours)

Characterization

- Nucleotide sequencing:
  - 198 primer sets for full genome sequencing (8 gene segments)
  - Sanger sequencing (ABI 3730xl)

H7 gene



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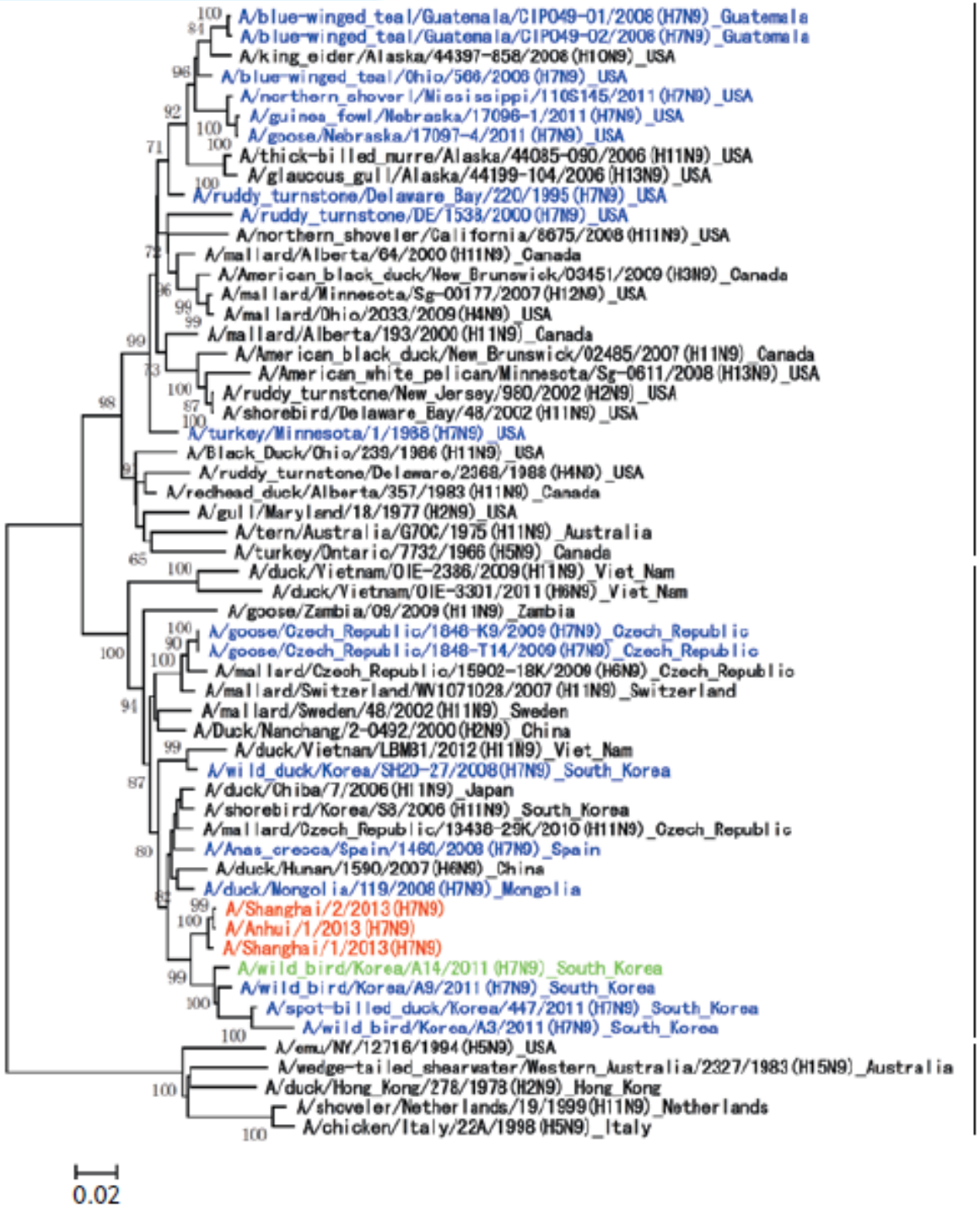
N9 gene



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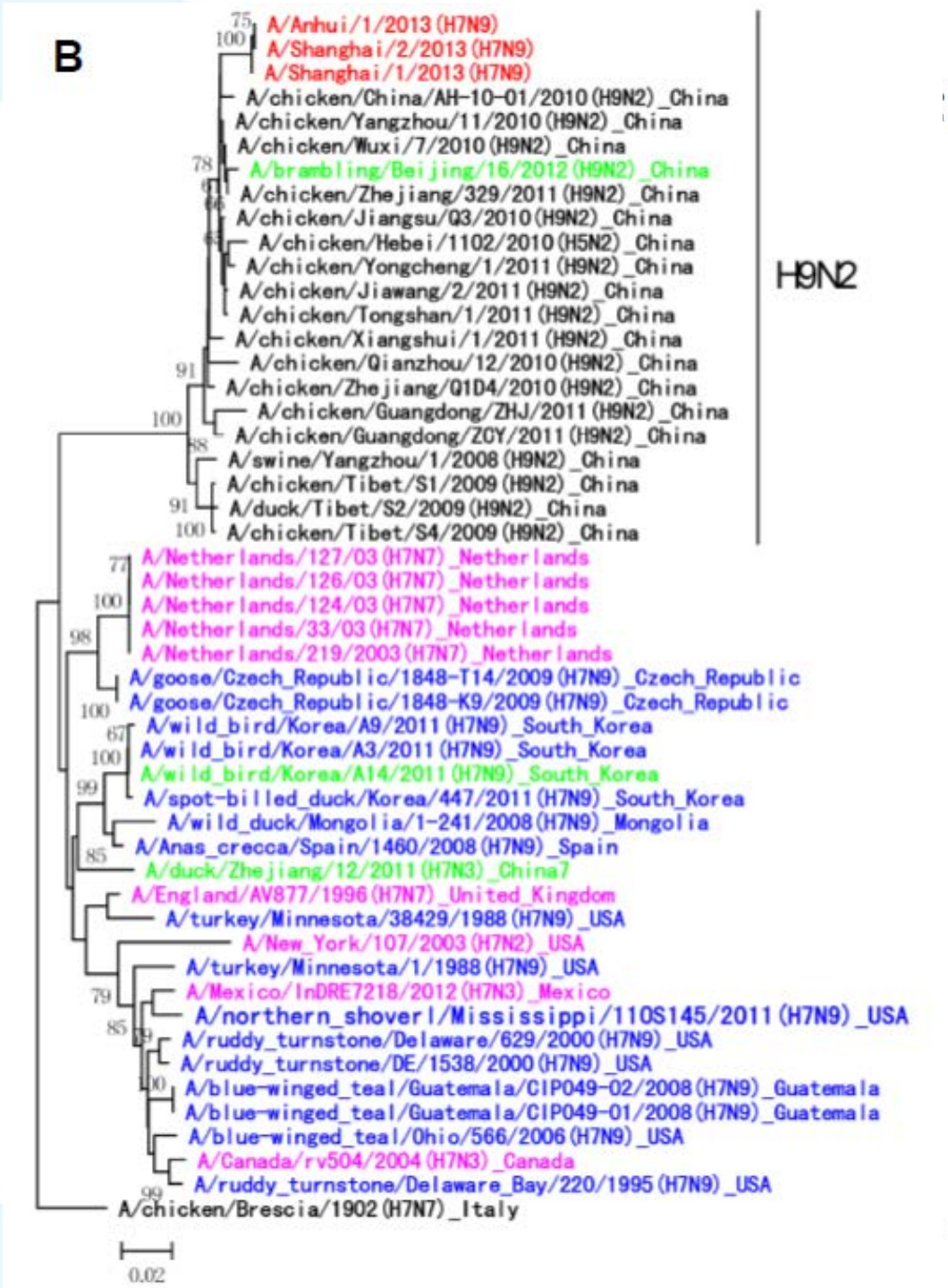
M gene



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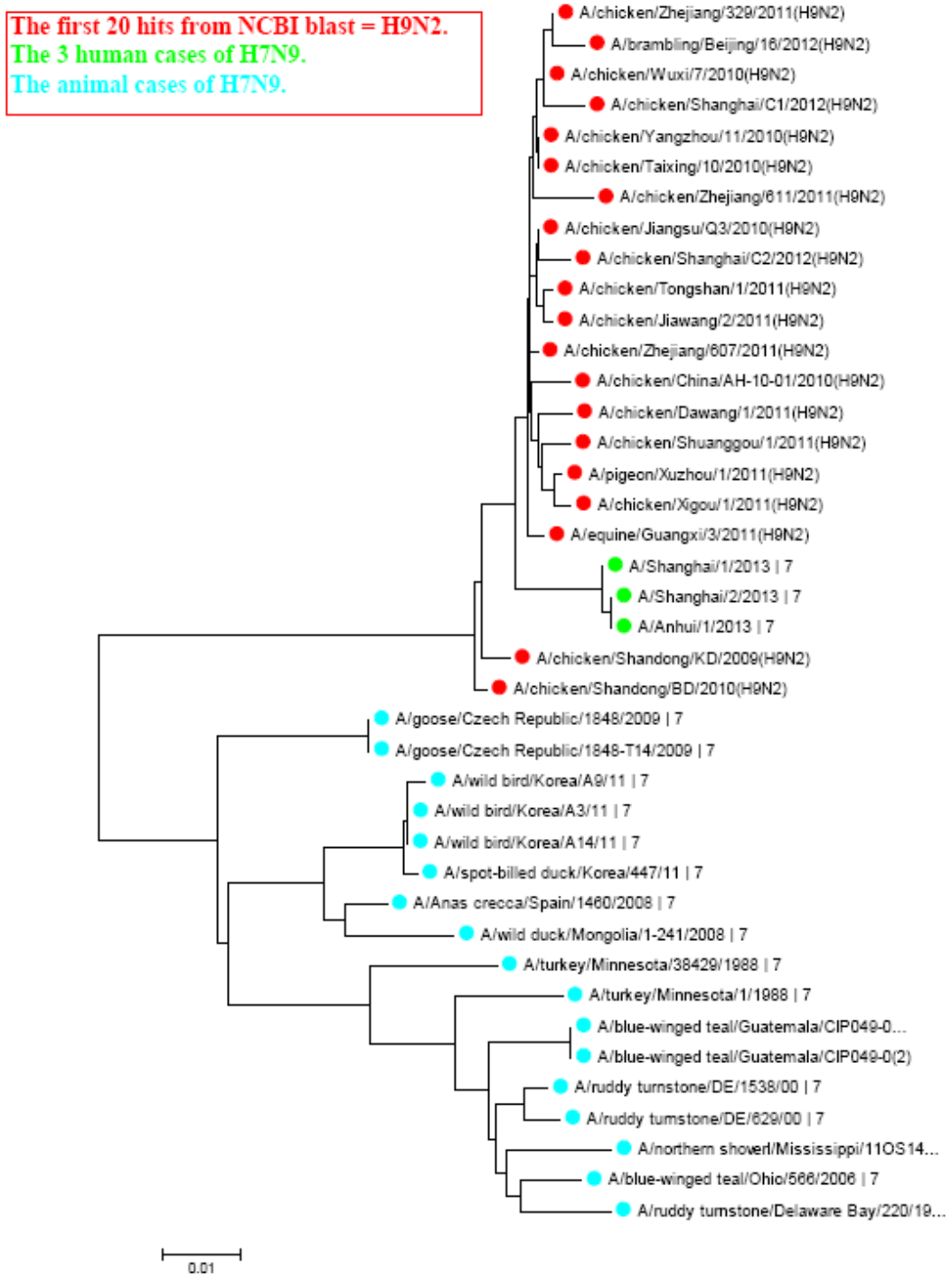
M gene


The first 20 hits from NCBI blast = H9N2.
The 3 human cases of H7N9.
The animal cases of H7N9.
A/chicken/Zhejiang/329/2011(H9N2)
A/brambling/Beijing/16/2012(H9N2)
A/chicken/Wuxi/7/2010(H9N2)
A/chicken/Shanghai/C1/2012(H9N2)
A/chicken/Yangzhou/11/2010(H9N2)
A/chicken/Taixing/10/2010(H9N2)
A/chicken/Zhejiang/611/2011(H9N2)
A/chicken/Jiangsu/Q3/2010(H9N2)
A/chicken/Shanghai/C2/2012(H9N2)
A/chicken/Tongshan/1/2011(H9N2)
A/chicken/Jiawang/2/2011(H9N2)
A/chicken/Zhejiang/607/2011(H9N2)
A/chicken/China/AH-10-01/2010(H9N2)
A/chicken/Dawang/1/2011(H9N2)
A/chicken/Shuanggou/1/2011(H9N2)
A/pigeon/Xuzhou/1/2011(H9N2)
A/chicken/Xigou/1/2011(H9N2)
A/equine/Guangxi/3/2011(H9N2)
A/Shanghai/1/2013 | 7
A/Shanghai/2/2013 | 7
A/Anhui/1/2013 | 7
A/chicken/Shandong/KD/2009(H9N2)
A/chicken/Shandong/BD/2010(H9N2)
A/goose/Czech Republic/1848/2009 | 7
A/goose/Czech Republic/1848-T14/2009 | 7
A/wild bird/Korea/A9/11 | 7
A/wild bird/Korea/A3/11 | 7
A/wild bird/Korea/A14/11 | 7
A/spot-billed duck/Korea/447/11 | 7
A/Anas crecca/Spain/1460/2008 | 7
A/wild duck/Mongolia/1-241/2008 | 7
A/turkey/Minnesota/38429/1988 | 7
A/turkey/Minnesota/1/1988 | 7
A/blue-winged teal/Guatemala/CIP049-0...
A/blue-winged teal/Guatemala/CIP049-0(2)
A/ruddy turnstone/DE/1538/00 | 7
A/ruddy turnstone/DE/629/00 | 7
A/northern shoveI/Mississippi/11OS14...
A/blue-winged teal/Ohio/566/2006 | 7
A/ruddy turnstone/Delaware Bay/220/19...
0.01

Hypothesis

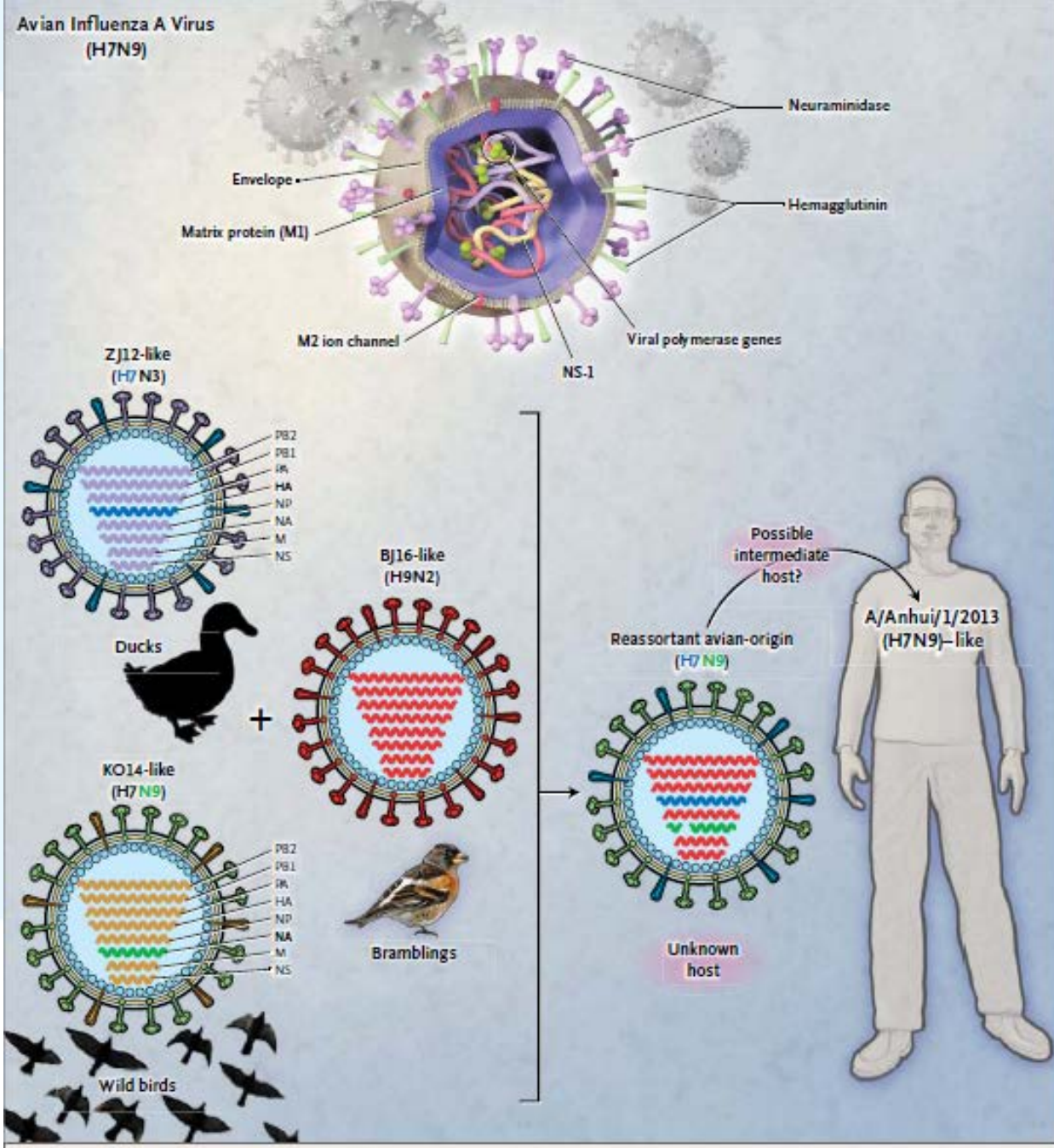


Figure 2. **Hypothetical Host and Lineage Origins of the Gene Segments of the Novel Reassortant Human Influenza A (H7N9) Viruses.**
The colors of the gene segments in the ovals indicate their origin. BJ16 denotes A/brambling/Beijing/16/2012, KO14 A/wild bird/Korea/A14/2011, and ZJ12 A/duck/Zhejiang/12/2011.

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TABLE 2

Nucleotide identity of novel influenza A(H7N9) virus genes and their closest relative, China, February–April 2013

Viral gene	Closest influenza virus relative	Nucleotide identity (%)
PB2	A/brambling/Beijing/16/2012(H9N2)	99
PB1	A/chicken/Jiangsu/Q3/2010(H9N2)	98
PA	A/brambling/Beijing/16/2012(H9N2)	99
HA	A/duck/Zhejiang/12/2011(H7N3)	95
NP	A/chicken/Zhejiang/611/2011(H9N2)	98
NA	A/mallard/Czech Republic/13438-29K/2010(H11N9)	96
M	A/chicken/Zhejiang/607/2011(H9N2)	98
NS	A/chicken/Dawang/1/2011(H9N2)	99

HA: haemagglutinin; M: matrix gene; NA: neuraminidase; NP: nucleoprotein; NS: non-structural gene; PA: RNA polymerase acidic subunit; PB1: RNA polymerase basic subunit 1; PB2: RNA polymerase basic subunit 2.

Characteristics of encoded proteins

- Haemagglutinin:
  - Cleavage site:
    - Single amino acid arginine, indicating low pathogenicity
  - Q226L in Anhui/1 and Shanghai/2:
    - Reduced binding to avian-like receptors (sialic acids with α-2,3 linkages to galactose) in human lower respiratory tract, and enhanced binding to mammalian-like α-2,6 receptors in human upper airway
    - Enabled transmission of HPAI H5N1 viruses by respiratory droplets in ferrets
  - T160A mutation at 150-loop:
    - Decreasing α-2,3 avian-like receptor binding

Characteristics of encoded proteins

- Neuraminidase:
  - Amino acids 69 to 73 deleted in stalk: Change in H5N1 tropism to respiratory tract, enhanced viral replication, and adaptation/transmission in domestic poultry; increased virulence in mice
  - R292K in A/Shanghai/1/2013, reported to be associated with in vitro reduced neuraminidase susceptibility (H4N2 in 1997; H1N9 in 1998)
- M2 protein:
  - S31N substitution: Resistance to amantadine

Characteristics of encoded proteins

- PB2 protein:
  - E627K mutation, associated with increased virulence in mice, mammalian adaptation and respiratory-droplet transmission of HPAI H5N1 virus in ferrets
- NS1 protein:
  - P42S, associated with increased virulence in mice

Testing development

- Availability of virus strain as positive control for laboratory testing
- Distribution of laboratory protocol on specific H7 real-time RT-PCR
- Distribution of viral RNA as positive control material for nucleic acid testing

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Rapid communications

GUIDING OUTBREAK MANAGEMENT BY THE USE OF INFLUENZA A(H7NX) VIRUS SEQUENCE ANALYSIS

M Jonges (marcel.jonges@rivm.nl)[1,2], A Meijer[1], R A Fouchier[2], G Koch[3], J Li[4], J C Pan[4], H Chen[5], Y L Shu[6], M P Koopmans[1,2]

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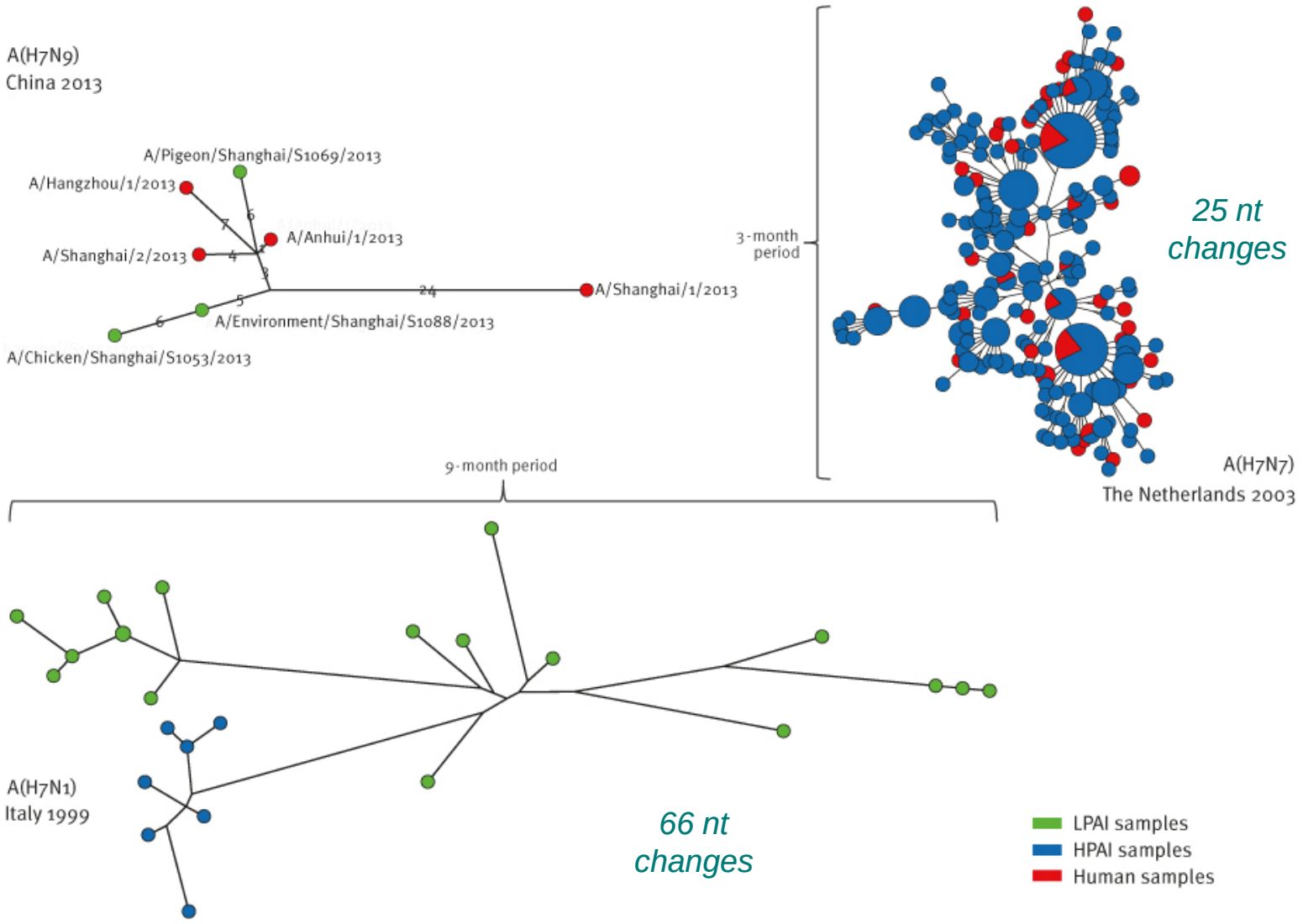
Date of submission: 15 April 2013

The recently identified human infections with avian influenza A(H7N9) viruses in China raise important questions

FIGURE

Genetic diversity of three influenza A(H7Nx) virus outbreaks expressed by minimum spanning trees



HPAI: highly pathogenic avian influenza; LPAI: low pathogenic avian influenza.

The minimum spanning trees were constructed using concatenated haemagglutinin, neuraminidase and PB2 (subunit of the influenza virus RNA polymerase complex) nucleotide sequences in BioNumerics software version 6.6.4. The scaling of the branches, representing nucleotide substitutions, is equal for the three outbreaks.

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Currently available tests

- Direct detection:
  - Antigen: Immunochromatography / immunofluorescence (not subtype-specific)
  - Nucleic acid
- Viral culture
- Serology (retrospective diagnosis)

Summary

- Virus of avian origin, present for certain duration with opportunities for genetic divergence
- Separate introduction to humans of genetically distinguishable virus strains
- Epidemiological and clinical suspicion for case finding and initiation of investigation
- Collection of proper and appropriate specimen for laboratory testing

衞生防護中心
Centre for Health Protection

Thank you

衞生署
Department of Health