

Laboratory Testing for Avian Influenza A(H7N9)



Patrick Tang, MD, PhD, FRCPC

Medical Microbiologist

BC Public Health Microbiology &
Reference Laboratory

Avian Influenza A(H7N9)

- First reported by China on March 31, 2013
 - Three laboratory-confirmed cases of a novel influenza A(H7N9) in Shanghai and Anhui
 - Illness onset between mid-February to mid-March
- Whole genome sequences posted on GISAID on same date
- NEJM paper released April 11, 2013

The NEW ENGLAND JOURNAL of MEDICINE

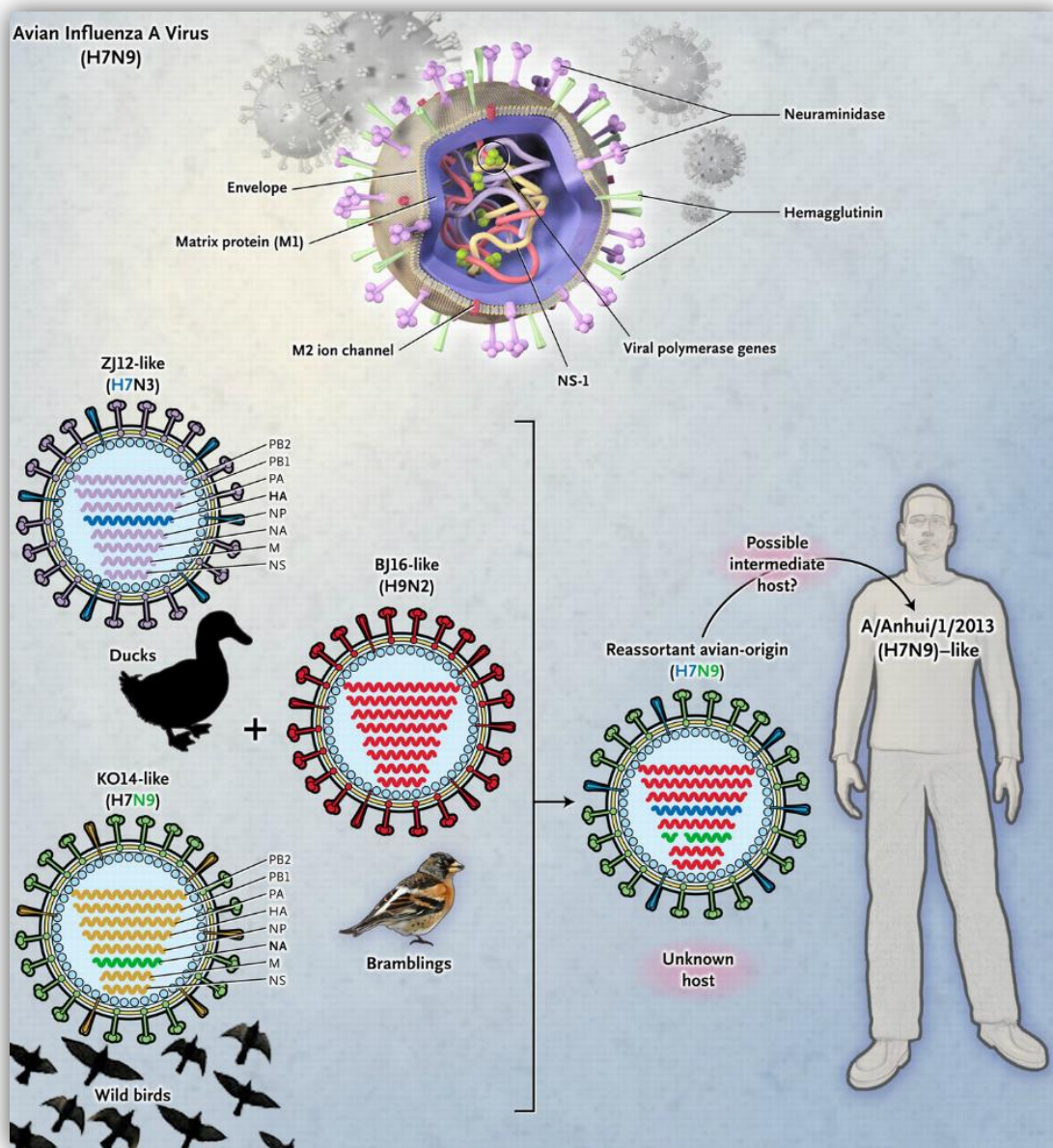
ORIGINAL ARTICLE

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., Xiyun 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.

Origins of Avian Influenza A(H7N9)

- Triple reassortant virus
 - HA most closely related to H7N3 from ducks in China in 2011
 - NA most closely related to H7N9 from wild birds in Korea in 2011
 - Internal components most closely related to H9N2 from chickens and bramblings in China sampled over past 5 years



Gao *et al.* Human Infection with a Novel Avian-Origin Influenza A (H7N9) Virus. **N Engl J Med** 2013.

Mutations Associated with Virulence

- Q226L mutation in HA
 - One of the mutations required for binding to mammalian receptors (α -2,6-sialic acids)
- E627K mutation in PB2
 - Associated with enhanced virulence in H5 viruses
- S31N mutation in M1
 - Adamantane resistance
- 15bp deletion in NA stalk
 - Suggests adaptation to chickens

April 29, 2013 H7N9 Update

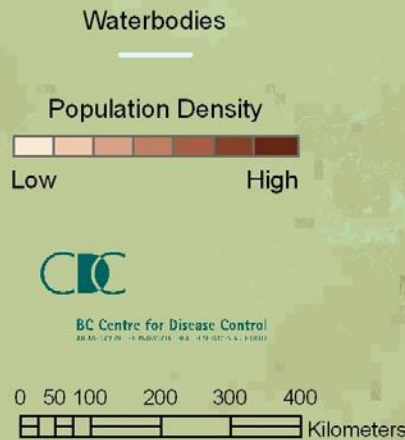
- 126 laboratory-confirmed cases
- 24 deaths
- Most infections and deaths in older adults
- Most cases in Zhejiang, Shanghai, Jiangsu and neighboring regions
 - Two cases in Beijing and one case in Taiwan (imported from Jiansu)
- Animal-to-person transmission
 - 60% had exposure to poultry
 - Chickens in live bird markets confirmed as one reservoir
 - No evidence for person-to-person transmission



H7N9 influenza activity in China/Taiwan by case residence, as of April 26th, 2013



Province	Deaths / Cases
Anhui	1 / 5
Beijing	0 / 2
Fujian	0 / 1
Henan	0 / 4
Jiangsu	4 / 26
Jiangxi	0 / 2
Shandong	0 / 1
Shanghai	11 / 30
Zhejiang	6 / 43
Taiwan	0 / 1
Total	23* / 115



Notes: *One death could not be attributed to a province of residence due to insufficient data.
 *City of residence for some cases not available. Taiwan case resides in Taiwan but works in Suzhou. Fatal Jiande case resided in Jiande but worked in Taicang. Data compiled from ProMed, GPHIN alerts and other public reports. Map created April 26th, 2013 by BCCDC.

Laboratory Testing (BC PHMRL)

- Severe respiratory infection work-up
 - Standard SRI workup as per local facility
 - May include testing for bacterial agents of atypical pneumonia
 - Consultation required for H7N9 testing
 - NP swab, throat swab, sputum or other respiratory samples as indicated (BAL, tracheal aspirate, etc.)
 - Testing for fluA/fluB/RSV by in-house multiplex RT-PCR
 - Negative → Luminex RVP multiplex PCR
 - Positive → subtyping for H1, H3, H1(pdm09) by RT-PCR

Laboratory Testing (BC PHMRL)

- H7N9-specific tests
 - Subtyping by real-time RT-PCR targeting HA gene
 - In-house designed assay
 - National Microbiology Laboratory (Canada) assay
 - Modification of original CDC assay for H7 subtyping
 - WHO/CNIC assay
 - Subtyping by real-time RT-PCR targeting NA gene
 - WHO/CNIC assay
 - Sequencing of HA, NA and M genes

Advanced Laboratory Testing

(BC PHMRL)

- Virus culture and isolation in MDCK cells in containment level-3 laboratory
- Whole genome sequencing of virus
 - Shotgun sequencing on Illumina MiSeq
 - PCR + Sanger sequencing



Sensitivity of Influenza A RT-PCR

- Most Flu A RT-PCR assays target the M1 gene
 - Chen Y *et al.* **J Clin Microbiol** 2011; 49(4): 1653
 - CDC Human Influenza Virus Real-Time RT-PCR
- **Sequence mismatch** between primers for M1 target from common human influenza A viruses versus the novel avian H7N9 virus
- Created *in vitro* transcribed H7N9 RNA
 - Synthesized the M1 gene segment, cloned into expression vector, generated negative-sense RNA
- **No loss of sensitivity** despite mismatch

Testing for Avian Influenza A(H7N9)

- Currently **all influenza A positive samples** will be **subtyped** for H1, H3, H1(pdm09) and H7
- If H7N9 cases are detected in North America and/or enhanced surveillance is required:
 - Incorporate H7 subtyping assay into our influenza screening assay OR perform stand-alone H7 RT-PCR assay as a front-line test
 - Sequencing of M gene to monitor mutations which may affect assay sensitivity

Questions?

