

# Sequence Changes Associated with Respiratory Transmission of H7N1 Influenza Virus in Mammals

**Terence S. Dermody**<sup>a,b,c</sup> Editor, *Journal of Virology*, **Rozanne M. Sandri-Goldin**<sup>d</sup> Editor-in-Chief, *Journal of Virology*, **Thomas Shenk**<sup>e</sup> Chair, ASM Journals Board

Departments of Pediatrics<sup>a</sup> and Pathology, Microbiology, and Immunology<sup>b</sup> and Elizabeth B. Lamb Center for Pediatric Research,<sup>c</sup> Vanderbilt University School of Medicine, Nashville, Tennessee, USA; Department of Microbiology and Molecular Genetics, University of California, Irvine, California, USA<sup>d</sup>; Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA<sup>e</sup>

In this issue of the *Journal of Virology* (JVI), we publish a paper by **Troy C. Sutton and colleagues** from the University of Maryland, Virginia-Maryland College of Veterinary Medicine, and Istituto Zooprofilattico Sperimentale delle Venezie in Padua, Italy, reporting sequence changes in highly pathogenic avian influenza virus A/H7N1 (A/H7N1) associated with airborne transmission in mammals (1). The authors found that serial inoculation of A/H7N1 into ferrets, housed under conditions allowing the transmission of virus by airborne routes but not by direct contact, led to the selection of variants capable of ferret-to-ferret spread. Sequence changes in the viral polymerase PB2 subunit, nucleoprotein (NP), matrix protein (M1), and hemagglutinin (HA) were found in A/H7N1 variants following the tenth and final passage. Importantly, these sequence changes were not associated with any loss in virulence. These findings raise the possibility that sequence polymorphisms in influenza virus internal proteins, in addition to those previously described for HA (2–4), regulate airborne transmission of highly pathogenic avian influenza virus strains in mammals.

Highly pathogenic avian influenza viruses have been sporadically introduced into humans with substantial morbidity and mortality (5–8). Although case fatality rates are not known with certainty, these strains are likely more virulent in humans than the 1918 influenza virus A/H1N1 pandemic strain (9). There are no reports of A/H7N1 transmission to humans, but the virulence of this strain in birds suggests that it could be similarly virulent in humans. The study by Sutton et al. (1) provides clues about the sequence changes that may allow airborne transmission of A/H7N1 in mammals. This information may stimulate additional work in the field to understand whether and how these sequences influence influenza virus transmission, which is a critical factor in the evolution of pandemic strains (10). Moreover, this new sequence information may improve public health surveillance for these sequence signatures, guide preparation of influenza vaccines, and lead to identification of new antiviral drug targets.

Given the potential concerns about enhancing the transmission of H7N1 influenza virus in mammals, the authors incorporated several features in the experimental design to mitigate risk (1). First, the A/ostrich/Italy/2332/2000 (H7N1) parental strain and the variants isolated in this study display avian ( $\alpha$ 2,3-linked sialic acid) and not human ( $\alpha$ 2,6-linked sialic acid) receptor-binding specificity. Second, the parental A/H7N1 virus is susceptible to oseltamivir and antigenically matched to an A/Netherlands/219/2003 (H7N7) experimental vaccine (11). Third, all experiments in this study were conducted in an enhanced animal biosafety level 3 laboratory appropriate for highly pathogenic avian influenza virus strains and routinely inspected by both in-

stitutional biosafety and United States Department of Agriculture officials.

Prior to submission of this paper to JVI, the study was evaluated for the possibility of dual use research of concern (DURC) by the University of Maryland Institutional Biosafety Committee (IBC) and the National Institute of Allergy and Infectious Diseases (NIAID). As described in the “United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern” (12), DURC is defined as “life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security” (12). Evaluation by both groups for the possibility of DURC consisted of three sequential steps, each asking a specific question. First, does the work involve one of the 15 agents and toxins specified by the U.S. government DURC policy? In this case, highly pathogenic avian influenza virus is one of the 15 agents and toxins. Second, does the work involve one of the seven listed experiments (or “effects”)? Again, in this case, enhancing transmission of A/H7N1 in mammals constitutes one of the seven effects. Third, does the resulting knowledge, information, products, or technologies meet the definition of DURC as defined in the policy? The University of Maryland IBC and NIAID differed about whether the study met the definition of DURC. However, neither group recommended against publication.

As is the case for all other papers considered for publication by JVI, the reviewers were asked to evaluate the paper by Sutton et al. for novelty, scientific rigor, and significance and to consider whether the research represented DURC. The manuscript also was evaluated for DURC by senior editors at the American Society for Microbiology, which publishes JVI. During this process, the authors, the University of Maryland IBC leadership, and NIAID representatives were asked for clarification about their adjudication of the DURC issue. Prior to reaching a final decision about publication, ASM convened a teleconference of the editors and officials from the NIH, who conveyed the perspective of the Department of Health and Human Services. The ASM editors did not

Published ahead of print 2 April 2014

Editor: M. J. Imperiale

Address correspondence to Terence S. Dermody, [terry.dermody@vanderbilt.edu](mailto:terry.dermody@vanderbilt.edu).

Copyright © 2014, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JVI.00886-14

reach consensus about whether the work represents DURC. However, it was concluded that the benefits of publishing the paper outweighed any potential risks, and ASM decided to move forward with publication.

As we have written previously (13), a designation of DURC should not necessarily preclude conduct of the research or communication of the findings. Consistent with our evaluation of a previous gain-of-function influenza virus study (4), we conducted a careful risk assessment of the report that appears in this issue of JVI that involved experts in influenza virus research, biosafety and biosecurity, and scientific publication and included affiliates of the U.S. Government. Following this consideration and our commitment to responsible publication, we concluded that the new information provided by Sutton et al. (1) represents an important contribution to an understanding of influenza virus transmission with the potential to limit human influenza illness.

We acknowledge that gain-of-function research on influenza virus is controversial (14–18). However, given the threat to human health posed by highly pathogenic avian influenza virus strains and the paucity of countermeasures available, we think that research on these viruses is important. We support efforts in the influenza virus research community to conduct this work to answer the most important scientific questions in the safest possible manner. Ongoing dialogue about these studies by the stakeholder communities, research institutions, funding agencies, and regulatory bodies will provide additional guidance about how this research can be most effectively communicated.

## REFERENCES

- Sutton T, Finch C, Shao H, Angel M, Chen H, Capua I, Cattoli G, Monne I, Perez DR. 2014. Airborne transmission of highly pathogenic H7N1 influenza in ferrets. *J. Virol.* 88:6623–6635. <http://dx.doi.org/10.1128/JVI.02765-13>.
- Imai M, Watanabe T, Hatta M, Das SC, Ozawa M, Shinya K, Zhong G, Hanson A, Katsura H, Watanabe S, Li C, Kawakami E, Yamada S, Kiso M, Suzuki Y, Maher EA, Neumann G, Kawaoka Y. 2012. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486:420–428. <http://dx.doi.org/10.1038/nature10831>.
- Herfst S, Schrauwen EJ, Linster M, Chutinimitkul S, de Wit E, Munster VJ, Sorrell EM, Bestebroer TM, Burke DF, Smith DJ, Rimmelzwaan GF, Osterhaus AD, Fouchier RA. 2012. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336:1534–1541. <http://dx.doi.org/10.1126/science.1213362>.
- Zaraket H, Bridges OA, Duan S, Baranovich T, Yoon SW, Reed ML, Salomon R, Webby RJ, Webster RG, Russell CJ. 2013. Increased acid stability of the hemagglutinin protein enhances H5N1 influenza virus growth in the upper respiratory tract but is insufficient for transmission in ferrets. *J. Virol.* 87:9911–9922. <http://dx.doi.org/10.1128/JVI.01175-13>.
- Subbarao K, Klimov A, Katz J, Regnery H, Lim W, Hall H, Perdue M, Swayne D, Bender C, Huang J, Hemphill M, Rowe T, Shaw M, Xu X, Fukuda K, Cox N. 1998. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science* 279:393–396. <http://dx.doi.org/10.1126/science.279.5349.393>.
- Claas EC, Osterhaus AD, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, Krauss S, Shortridge KF, Webster RG. 1998. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. *Lancet* 351:472–477. [http://dx.doi.org/10.1016/S0140-6736\(97\)11212-0](http://dx.doi.org/10.1016/S0140-6736(97)11212-0).
- Chen Y, Liang W, Yang S, Wu N, Gao H, Sheng J, Yao H, Wo J, Fang Q, Cui D, Li Y, Yao X, Zhang Y, Wu H, Zheng S, Diao H, Xia S, Zhang Y, Chan KH, Tsoi HW, Teng JL, Song W, Wang P, Lau SY, Zheng M, Chan JF, To KK, Chen H, Li L, Yuen KY. 2013. Human infections with the emerging avian influenza A H7N9 virus from wet market poultry: clinical analysis and characterisation of viral genome. *Lancet* 381:1916–1925. [http://dx.doi.org/10.1016/S0140-6736\(13\)60903-4](http://dx.doi.org/10.1016/S0140-6736(13)60903-4).
- Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, Chen J, Jie Z, Qiu H, Xu K, Xu X, Lu H, Zhu W, Gao Z, Xiang N, Shen Y, He Z, Gu Y, Zhang Z, Yang Y, Zhao X, Zhou L, Li X, Zou S, Zhang Y, Li X, Yang L, Guo J, Dong J, Li Q, Dong L, Zhu Y, Bai T, Wang S, Hao P, Yang W, Zhang Y, Han J, Yu H, Li D, Gao GF, Wu G, Wang Y, Yuan Z, Shu Y. 2013. Human Infection with a novel avian-origin influenza A (H7N9) virus. *N. Engl. J. Med.* 368:1888–1897. <http://dx.doi.org/10.1056/NEJMoa1304459>.
- Johnson NP, Mueller J. 2002. Updating the accounts: global mortality of the 1918–1920 “Spanish” influenza pandemic. *Bull. Hist. Med.* 76:105–115. <http://dx.doi.org/10.1353/bhm.2002.0022>.
- Morse SS, Mazet JA, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrel C, Lipkin WI, Daszak P. 2012. Prediction and prevention of the next pandemic zoonosis. *Lancet* 380:1956–1965. [http://dx.doi.org/10.1016/S0140-6736\(12\)61684-5](http://dx.doi.org/10.1016/S0140-6736(12)61684-5).
- Gambaryan AS, Matrosovich TY, Philipp J, Munster VJ, Fouchier RA, Cattoli G, Capua I, Krauss SL, Webster RG, Banks J, Bovin NV, Klenk HD, Matrosovich MN. 2012. Receptor-binding profiles of H7 subtype influenza viruses in different host species. *J. Virol.* 86:4370–4379. <http://dx.doi.org/10.1128/JVI.06959-11>.
- National Institutes of Health. 2012. United States Government policy for oversight of life sciences dual use research of concern. <http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/dual-use-research-concern>. Accessed 7 April 2014.
- Dermoddy TS, Sandri-Goldin RM, Shenk T. 2013. A new determinant of H5N1 influenza virus pathogenesis in mammals. *J. Virol.* 87:4795–4796. <http://dx.doi.org/10.1128/JVI.00474-13>.
- Fouchier RA, Kawaoka Y, Cardona C, Compans RW, Fouchier RA, Garcia-Sastre A, Govorkova EA, Guan Y, Herfst S, Kawaoka Y, Orenstein WA, Peiris JS, Perez DR, Richt JA, Russell C, Schultz-Cherry SL, Smith DJ, Steel J, Tompkins SM, Topham DJ, Treanor JJ, Tripp RA, Webby RJ, Webster RG. 2013. Avian flu: gain-of-function experiments on H7N9. *Nature* 500:150–151. <http://dx.doi.org/10.1038/500150a>.
- Fouchier RA, Kawaoka Y, Cardona C, Compans RW, Garcia-Sastre A, Govorkova EA, Guan Y, Herfst S, Orenstein WA, Peiris JS, Perez DR, Richt JA, Russell C, Schultz-Cherry SL, Smith DJ, Steel J, Tompkins SM, Topham DJ, Treanor JJ, Tripp RA, Webby RJ, Webster RG. 2013. Gain-of-function experiments on H7N9. *Science* 341:612–613. <http://dx.doi.org/10.1126/science.1243325>.
- Lipsitch M. 2013. Avian influenza: ferret H7N9 flu model questioned. *Nature* 501:33. <http://dx.doi.org/10.1038/501033e>.
- Mahmoud A. 2013. Gain-of-function research: unproven technique. *Science* 342:310–311. <http://dx.doi.org/10.1126/science.342.6156.310-b>.
- Rey F, Schwartz O, Wain-Hobson S. 2013. Gain-of-function research: unknown risks. *Science* 342:311. <http://dx.doi.org/10.1126/science.342.6156.311-a>.