Avian Influenza A(H7N9) Virus Infection in 2 Travelers Returning from China to Canada, January 2015

Danuta M. Skowronski, Catharine Chambers, Reka Gustafson, Dale B. Purych, Patrick Tang, Nathalie Bastien, Mel Krajden, Yan Li

In January 2015, British Columbia, Canada, reported avian influenza A(H7N9) virus infection in 2 travelers returning from China who sought outpatient care for typical influenza-like illness. There was no further spread, but serosurvey findings showed broad population susceptibility to H7N9 virus. Travel history and timely notification are critical to emerging pathogen detection and response.

Since February 2013, >600 human cases of avian influenza A(H7N9) infection have been reported from eastern China, where the virus is considered enzootic in poultry (1). Travel-associated cases have previously been reported in Asia (2); however, in January 2015, Canada reported 2 travel-associated cases, which are described here.

The Study
A married couple, both 56 years of age, from British Columbia (BC), Canada, traveled in Hong Kong (December 29, 2014–January 3, 2015, and January 6–7); Taipei, Taiwan (January 3–6); and Fujian Province, China (January 7–11), returning home on January 12. They recalled seeing live poultry and copious droppings while visiting Fujian on January 8 but recollected no other poultry contact.

Around January 3–7, the previously healthy woman experienced mild cough, sore throat, and hoarseness. She recovered, but influenza-like illness (ILI), including fever, cough, myalgia, and fatigue, developed on January 14. On January 15, she sought outpatient care. A healthcare worker (HCW) collected a nasal swab specimen, which was sent to the BC Public Health Microbiology and Reference Laboratory (BC-PHMRL), where most influenza testing is centralized in BC. On January 16, the sample tested positive for influenza A virus by reverse transcription PCR (RT-PCR), and the HCW was informed; the next business day (January 19), the patient was prescribed oseltamivir.

For surveillance purposes, BC-PHMRL conducts subtyping of all detected influenza A viruses. Despite a high virus titer (cycle threshold [Ct] 23.43), the specimen could not be subtyped for human influenza H1 or H3 virus by matrix gene–based RT-PCR. Further subtyping using RT-PCR–based targets for the hemagglutinin gene indicated an H7 virus. Sequence analysis of a matrix gene fragment showed 99% identity with H7N9 and H9N2 viruses, the latter of which is known to have donated internal genes to H7N9 virus (1,3). On January 23, BC-PHMRL notified public health authorities of a presumptive diagnosis of H7N9 virus infection in the woman (index case), and on January 26, Canada’s National Microbiology Laboratory (NML) confirmed the diagnosis by RT-PCR.

On January 13, a day before the woman became ill, her husband, who had a history of asthma, had onset of ILI symptoms (fever, productive cough, chest pain, dyspnea, headache, myalgia, and fatigue) and visited the same HCW. The HCW prescribed doxycycline but did not collect a specimen. On January 19, after his wife received a diagnosis of influenza, the man was prescribed oseltamivir. A throat swab specimen collected from him on January 23 was RT-PCR–positive for influenza A (Ct 29.79). On January 29, NML confirmed H7N9 virus infection.

Neither patient experienced conjunctivitis, which has been reported with H7N7 and H7N3 infections (3,4), and both recovered in self-isolation at home. Follow-up respiratory specimens were still positive by RT-PCR on January 26 (day 12 after ILI onset for the woman [Ct 33.80]; day 13 for the man [Ct 33.80]) and on January 28 (day 15 after ILI onset) for the man (Ct 30.21).

The HCW remained asymptomatic; however, because of the patients’ travel history, the HCW began oseltamivir prophylaxis after learning the index patient had laboratory-confirmed influenza. Throat swab specimens collected from the HCW on January 26 were influenza RT-PCR–negative. Approximately 20 other close contacts of the patients were placed under 10-day surveillance (from last exposure), including 1 who received oseltamivir prophylaxis.

Preliminary results from the population serosurvey were presented at the CACMID-AMMI Canada 2014 Annual Conference, April 3–5, 2014, Victoria, British Columbia, Canada.

DOI: http://dx.doi.org/10.3201/eid2201.151330

Author affiliations: British Columbia Centre for Disease Control, Vancouver, British Columbia, Canada (D.M. Skowronski, C. Chambers, P. Tang, M. Krajden); University of British Columbia, Vancouver (D.M. Skowronski, R. Gustafson, D.B. Purych, P. Tang, M. Krajden); Vancouver Coastal Health Authority, Vancouver (R. Gustafson); LifeLabs Medical Laboratories, Surrey, British Columbia, Canada (D.B. Purych); Public Health Agency of Canada, Winnipeg, Manitoba, Canada (N. Bastien, Y. Li); University of Manitoba, Winnipeg (Y. Li)
All contacts remained asymptomatic. Passengers on the flight taken by the patients while they were asymptomatic were not included in active surveillance because >10 days had elapsed (5); however, media communications included flight details and public health advice.

Virus cultures in Madin-Darby canine kidney cells were attempted with all patient samples; only the woman’s January 15 sample was culture-positive for influenza virus (A/British Columbia/1/2015[H7N9]). Phylogenetic analysis of the hemagglutinin and neuraminidase genes showed that the strain clustered with 2014 and 2015 H7N9 human isolates from Jiangsu, Zhejiang, and Fujian Provinces, China, and 2014 chicken isolates from Jiangxi, China, belonging to clade W2-C (1). Similar to genomes of previous human H7N9 isolates, the genome of the patient’s isolate showed clinically relevant markers: substitutions G186V (H3 numbering), Q226L, and T160L in the hemagglutinin for increased human receptor affinity; substitution E627K in polymerase basic 2 for mammalian replication; substitutions S31N and V27I in matrix 2 for amantadine resistance; deletion in the neuraminidase stalk (positions 69–73); and neuraminidase inhibitor susceptibility (3).

Antibody titers to H7N9 and recent human influenza A(H3N2) and A(H1N1)pdm09 strains in paired serum samples from the case-patients and HCW were measured by hemagglutination inhibition assay at NML (Table 1) (6). At ≈7 weeks after ILI onset, the case-patients showed seroconversion (>4-fold antibody rise) to H7N9 virus; the HCW had no detectable H7N9 antibody (Table 1).

As part of population risk assessment, we also previously measured hemagglutination inhibition antibody titers to H7N9 virus in anonymized residual serum samples collected and banked from patients attending community-based laboratory test sites in 2010 (n = 1,116; ≥100 samples/10-year age group) (7) and 2013 (n = 496; ≥50 samples/10-year age group) (8) across the same BC region to which the couple returned. The assessment was conducted as described (7,8) and approved by the University of British Columbia Research Ethics Board. Results showed broad population serosusceptibility to H7N9 virus: 5% (10/201) of serum samples collected from patients ≥60 years of age in 2013 had low-level titers (≥10 but <40), but no other samples had detectable H7N9 virus antibody (Table 2). Three samples with titers ≥20 showed titers <10 by microneutralization assay (Table 2).

Conclusions
The onset of ILI in 2 BC patients 5–6 days after observing poultry in China is consistent with the median 5-day incubation period reported elsewhere for H7N9 virus (9) and with common-source acquisition of the virus in Fujian. However, we cannot rule out other unrecognized exposures or person-to-person transmission between the couple. Continued RT-PCR detection in respiratory specimens 2 weeks after ILI onset has been documented (9) but does not necessarily signify ongoing shedding of infectious virus. Although previous case series have reported inconsistent antibody responses to H7N9 virus, often with low avidity and potentially correlated with clinical outcome (10–12), both BC case-patients with typical outpatient ILI demonstrated seroconversion.

Reported human cases of H7N9 infection have mostly been in older men, and two thirds have been categorized as severe (2,3,9). Mild illness has occasionally

### Table 1. Antibody titers to avian influenza A/H7N9 virus and recent human influenza A(H3N2) and A(H1N1)pdm09 virus strains for 2 persons with virologically confirmed H7N9 virus infection and for an HCW contact, British Columbia, Canada, January 2015

| Person, date of specimen collection | Duplicate inverse HI titers and GMT for influenza antibody | A/British Columbia/1/2015[H7N9]† | A/Switzerland/9715293/2013[H3N2]‡§ | A/California/07/2009(H1N1)¶ | GMT |
|-------------------------------------|----------------------------------------------------------|----------------------------------|----------------------------------|----------------------------------|-----|
| **Index patient#**                  |                                                          | Titer 1 | Titer 2 | GMT  | Titer 1 | Titer 2 | GMT |
| January 26                          | 20                                                       | 20      | 20     | 10    | 10      | 10     | 10  |
| March 5                             | 80                                                       | 160     | 113    | 10    | 10      | 10     | 10  |
| **Second patient#**                 |                                                          | Titer 1 | Titer 2 | GMT  | Titer 1 | Titer 2 | GMT |
| January 26                          | 20                                                       | 40      | 28     | 10    | 10      | 10     | 10  |
| March 5                             | 160                                                      | 160     | 113    | 20    | 20      | 20     | 20  |
| **HCW contact**                     |                                                          | Titer 1 | Titer 2 | GMT  | Titer 1 | Titer 2 | GMT |
| January 27                          | <10                                                      | <10     | <10    | 20    | 20      | 20     | 20  |
| May 1                               | <10                                                      | <10     | <10    | 40    | 40      | 40     | 40  |

*The 2 H7N9 virus–infected persons were a married couple; the woman was the index patient, and the man was the second patient. GMT, geometric mean titer; HCW, healthcare worker; HI, hemagglutination inhibition.
†Assay was conducted by using homologous H7N9 virus isolated from the index patient (Global Initiative on Sharing Avian Influenza Data accession no. EPI_ISL_171342); the virus was antigenically equivalent to influenza A/Anhui/1/2013(H7N9), which was used in the population serosurvey reported in Table 2. The HI assay was conducted by using horse erythrocytes, as previously described (6).
‡Assay was conducted by using viruses of each human influenza A H1 and H3 subtype to which strains identified globally during the 2014–15 influenza season were considered antigenically related (see http://www.who.int/influenza/vaccines/virus/recommendations/2015_16_north/en/). Titers were measured according to standard assay protocols of the National Microbiology Laboratory, Canada’s influenza reference laboratory.
§Assay was conducted by using guinea pig erythrocytes and in the presence of oseltamivir carboxylate to address potential neuraminidase-mediated binding of influenza A(H3N2) viruses to erythrocytes.
¶Assay was conducted by using turkey erythrocytes.
##Received neither the 2013–14 nor 2014–15 influenza vaccine nor prior pneumococcal vaccine.
**Received the 2013–14 and the 2014–15 influenza vaccines.
been reported in children (2, 13, 14), but, as exemplified by the BC cases, adults can also experience milder infection. Imported cases of novel influenza are less likely to be recognized if they are mild. In that regard, identification of H7N9 virus in an outpatient setting was adventitious. Travel history triggered specimen collection by the HCW, and identification of nonsubtypeable influenza by the provincial laboratory prompted further investigation and public health notification.

Despite broad susceptibility and instances of household or familial transmission, H7N9 virus has not demonstrated easy person-to-person spread. Poultry exposure remains the major risk factor for human H7N9 infection (2, 3, 9). Primary prevention messages should emphasize to travelers that they avoid exposure to poultry and uncooked poultry products while visiting affected areas. As illustrated by a prior imported case of avian influenza A(H5N1) virus to Alberta, Canada, however, such exposures may not always be recognized or avoidable (15). Screening should therefore begin with travel history in the 2 weeks before onset of acute respiratory illness. Patients should be encouraged to volunteer recent travel histories, and HCWs should elicit information regarding travel to affected areas. Public health and laboratory partners should be notified of suspect cases, as appropriate, during the diagnostic work-up, so that emerging pathogen screening, risk assessment, and advice can be guided in a timely manner.

Acknowledgments
We acknowledge the 2 patients and healthcare worker, who generously shared their experiences. We also thank Suzana Sabaiduc for assistance with manuscript preparation.

Funding in support of the population serosurvey was provided by the Michael Smith Foundation for Health Research (grant OT-GIA-00012091).

D.P. was previously a shareholder with BC Biomedical Laboratories, which contributed anonymized serum included in serosurveys, but he did not receive direct compensation for contributions to this analysis. M.K. has received research grants from Roche, Merck, Gen-Probe, Boehringer Ingelheim, and Siemens.

Dr. Skowronski is an epidemiologist at the British Columbia Centre for Disease Control, where she is responsible for surveillance, rapid response research, and policy advice related to influenza and other emerging or reemerging respiratory pathogens. Her primary research interests are influenza molecular and immunopathogenesis and influenza vaccine effectiveness and safety evaluation.

References
1. Lam TT, Zhou B, Wang J, Chai Y, Shen Y, Chen X, et al. Dissemination, divergence and establishment of H7N9 influenza viruses in China. Nature. 2015;522:102–5.
2. European Centre for Disease Prevention and Control. Human infection with avian influenza A(H7N9) virus. Fourth update, February 2, 2015 [cited 2015 Jul 23]. http://ecdc.europa.eu/en/publications/Publications/RRA-Influenza-A-H7N9-update-four.pdf
3. To KKW, Chan JFW, Chen H, Li L, Yuen K-Y. The emergence of influenza A H7N9 in human beings 16 years after influenza A H5N1: a tale of two cities. Lancet Infect Dis. 2013;13:809–21. http://dx.doi.org/10.1016/S1473-3099(13)70167-1
4. Skowronski DM, Li Y, Tweed SA, Tam TWS, Petric M, David ST, et al. Protective measures and human antibody response during an avian influenza H7N3 outbreak in poultry in British Columbia, Canada. CMAJ. 2007;176:47–53. http://dx.doi.org/10.1503/cmaj.060204
5. European Centre for Disease Prevention and Control. Risk assessment guidelines for infectious diseases transmitted on aircraft
6. Centers for Disease Control and Prevention. Modified hemagglutination-inhibition (HI) assay using horse RBCS for serologic detection of antibodies to H7 subtype avian influenza virus in human sera. Version 1 [cited 2015 July 23]. https://consise.tghn.org/site_media/media/articles/160713_Modified_Hemagglutination_Inhibition_Assay_Using_Horse_RBCs.pdf

7. Skowronski DM, Janjua NZ, De Serres G, Purych D, Gilca V, Scheifele DW, et al. Cross-reactive and vaccine-induced antibody to an emerging swine-origin variant of influenza A virus subtype H3N2 (H3N2)v. J Infect Dis. 2012;206:1852–61. http://dx.doi.org/10.1093/infdis/jis350

8. Skowronski DM, Chambers C, Sabaiduc S, Janjua NZ, Li G, Petric M, et al. Pre- and postpandemic estimates of 2009 pandemic influenza A(H1N1) seroprotection to inform surveillance-based incidence by age, during the 2013–2014 epidemic in Canada. J Infect Dis. 2015;211:109–14. http://dx.doi.org/10.1093/infdis/jiu366

9. Gao H-N, Lu H-Z, Cao B, Du B, Shang H, Gan J-H, et al. Clinical findings in 111 cases of influenza A (H7N9) virus infection. N Engl J Med. 2013;368:2277–85. http://dx.doi.org/10.1056/NEJMoa1305584

10. Zhang A, Huang Y, Tian D, Lau EH, Wan Y, Liu X, et al. Kinetics of serological responses in influenza A(H7N9)-infected patients correlate with clinical outcome in China, 2013. Euro Surveill. 2013;18:20657.

11. Yang S, Chen Y, Cui D, Yao H, Lou J, Huo Z, et al. Avian-origin influenza A(H7N9) infection in influenza A(H7N9)-affected areas of China: a serological study. J Infect Dis. 2014;209:265–9. http://dx.doi.org/10.1093/infdis/jit430

12. Guo L, Zhang X, Yu X, Chen L, Zhou H, Gao X, et al. Human antibody responses to avian influenza A(H7N9) virus, 2013. Emerg Infect Dis. 2014;20:192–200. http://dx.doi.org/10.3201/eid2012.1311094

13. Chen Z, Liu H, Lu J, Luo L, Li K, Liu Y, et al. Asymptomatic, mild, and severe influenza A(H7N9) virus infection in humans, Guangzhou, China. Emerg Infect Dis. 2014;20:1535–40. http://dx.doi.org/10.3201/eid2009.140424

14. Ip DK, Liao Q, Wu P, Gao Z, Cao B, Feng L, et al. Detection of mild to moderate influenza A/H7N9 infection by China’s national sentinel surveillance system for influenza-like illness: case series. BMJ. 2013;346:f3693. http://dx.doi.org/10.1136/bmj.f3693

15. Pabbaraju K, Tellier R, Wong S, Li Y, Bastien N, Tang JW. Full-genome analysis of avian influenza A(H5N1) virus from a human, North America, 2013. Emerg Infect Dis. 2014;20:887–91. http://dx.doi.org/10.3201/eid2005.140164

Address for correspondence: Danuta M. Skowronski, British Columbia Centre for Disease Control, 655 W 12th Ave, Vancouver, BC V5Z 4R4, Canada; email: danuta.skowronski@bccdc.ca