Live Animal Markets in Minnesota: A Potential Source for Emergence of Novel Influenza A Viruses and Interspecies Transmission

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(See the Editorial Commentary by Webby on pages 1363–4.)

Background. Live animal markets have been implicated in transmission of influenza A viruses (IAVs) from animals to people. We sought to characterize IAVs at 2 live animal markets in Minnesota to assess potential routes of occupational exposure and risk for interspecies transmission.

Methods. We implemented surveillance for IAVs among employees, swine, and environment (air and surfaces) during a 12-week period (October 2012–January 2013) at 2 markets epidemiologically associated with persons with swine-origin IAV (variant) infections. Real-time reverse transcription polymerase chain reaction (rRT-PCR), viral culture, and whole-genome sequencing were performed on respiratory and environmental specimens, and serology on sera from employees at beginning and end of surveillance.

Results. Nasal swabs from 11 of 17 (65%) employees tested positive for IAVs by rRT-PCR; 7 employees tested positive on multiple occasions and 1 employee reported influenza-like illness. Eleven of 15 (73%) employees had baseline hemagglutination inhibition antibody titers ≥40 to swine-origin IAVs, but only 1 demonstrated a 4-fold titer increase to both swine-origin and pandemic A/Mexico/4108/2009 IAVs. IAVs were isolated from swine (72/84), air (30/45), and pen railings (5/21). Whole-genome sequencing of 122 IAVs isolated from swine and environmental specimens revealed multiple strains and subtype codetections. Multiple gene segment exchanges among and within subtypes were observed, resulting in new genetic constellations and reassortant viruses. Genetic sequence similarities of 99%–100% among IAVs of 1 market customer and swine indicated interspecies transmission.

Conclusions. At markets where swine and persons are in close contact, swine-origin IAVs are prevalent and potentially provide conditions for novel IAV emergence.

Keywords. influenza; human; swine; live animal markets.

Variant influenza viruses are swine-origin influenza A viruses (IAVs) that infect humans [1]. During 2012, an outbreak of variant influenza A (H3N2v) virus sickened 309 persons in 12 states [2]. Multiple patients with H3N2v reported having swine exposure at agricultural fairs, a setting associated with previous cases of variant influenza [3–8]. In Minnesota, the first 2 variant IAV infections reported during 2012 occurred among live animal market customers. These 2 cases were the latest in a series of variant influenza cases associated with exposure to swine at live animal markets in Minnesota [9].

Live animal markets have been implicated in the interspecies transmission of IAV. Specifically, in China, markets that sell poultry have been linked to human cases of illness due to H7N9 [10] and H5N1 IAV infections [11]. However, limited information is available

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about the risk for interspecies transmission and sources of exposure of IAVs at live animal markets in the United States. Studies of other swine environments have isolated IAVs from air samples [12] and demonstrated IAV transmission through fomites [13]. Because swine can support exchange of genetic material among avian, swine, and human IAVs [14], they can be sources of novel influenza viruses, which can have pandemic potential.

We conducted surveillance among employees, swine, and of the environment for IAVs at 2 live animal markets in Minnesota that sell swine and have been epidemiologically associated with variant influenza cases. Our objectives were to characterize IAVs at these markets, assess potential routes for occupational exposure, and evaluate the risk for interspecies transmission.

METHODS

Ethical Considerations

This investigation was considered a public health response by the Minnesota Department of Health (MDH) Institutional Review Board. Participation was voluntary; written informed consent was obtained from participants. Swine and environmental sampling was conducted according to the University of Minnesota (UMN) Institutional Animal Care and Use Committee and Institutional Biosafety Committee–approved protocols.

Setting

Surveillance for IAVs was conducted at 2 live animal markets (markets A and B) in St Paul, Minnesota, for a 12-week period between 8 October 2012 and 12 January 2013. The markets serve a diverse ethnic clientele with mostly Hmong, Somali, Ethiopian, and Latino customers. At market A, animals were housed in a single indoor holding area, with goats and sheep in one pen and swine in another. At market B, swine were kept separately from other animals inside a holding area segregated in pens by size. Chickens, ducks, sheep, goats, and cattle were held in attached rooms. None of these animal species were included in the surveillance. Both markets purchase cross-bred swine with unknown IAV vaccination status from multiple livestock suppliers multiple times per week. These livestock suppliers are sourced with pigs from multiple origins, ages, and health status. Swine are held until sold, and the facilities are rarely empty. Swine were approximately 3–6 months old, and each market sells approximately 80–200 swine per week.

Customers enter animal holding areas to choose swine that are butchered and processed by market employees inside the animal processing area. Customers receive processed meat inside the customer area and use nearby sinks to clean the meat and animal byproducts.

Employee Surveillance

We conducted a prospective cohort study among market employees. Employee participation was voluntary and after enrollment, participants completed a baseline questionnaire to assess chronic medical conditions, influenza vaccination status, length of employment, and job duties. Each week, we asked study participants whether they had experienced any symptoms of influenza-like illness during the preceding week, about changes in their job duties, and whether they used personal protective equipment (PPE) when performing job duties. Available PPE included surgical face masks, rubber gloves, and boots.

Nasal swabs, collected weekly from employees, were tested at MDH by using the Centers for Disease Control and Prevention (CDC) human IAV real-time reverse transcription polymerase chain reaction (rRT-PCR) diagnostic panel [15, 16], and viral culture (primary rhesus monkey kidney cells; Diagnostic Hybrids, Inc, Athens, Ohio) [17]. Real-time RT-PCR cycle threshold (Ct) values <38 were considered positive, and positive samples were submitted to the CDC for retesting. Paired serum samples were collected from employees within 7 weeks of enrollment and at week 12, and sent to the CDC for serologic testing. We measured antibody titers by hemagglutination inhibition (HI) assays by using 0.5% turkey erythrocytes and microneutralization assays (MN) by using Madin-Darby canine kidney (MDCK) cells [17] with viruses as follows: (1) A/swine/Minnesota/A01381005/2012 and A/swine/Minnesota/A01381006/2012 (H1N2 viruses containing classical swine lineage hemagglutinin [HA] isolated from the markets); (2) A/Ohio/13/2012 (an H3N2v, genetically similar to swine H3N2 viruses circulating at the markets); (3) A/Minnesota/14/2012 (an H1N2v virus with former seasonal HA lineage); (4) A/Victoria/361/2011 (seasonal H3N2); and (5) A/Mexico/4108/2009 (H1N1pdm09), an A/California/07/2009–like virus. Northern hemisphere 2012–2013 seasonal vaccines included A/Victoria/361/2011–like and A/California/07/2009–like viruses. A ≥4-fold increase in antibody titer by either assay was considered evidence of IAV infection. A baseline HI titer ≥40 was considered a seropositive result.

Swine Surveillance

Once a week, oral fluids (1–5 samples/market/week) were collected by hanging cotton ropes inside swine pens and letting swine chew the ropes for 30 minutes [18]. Once a week, lung tissue specimens from slaughtered swine (2–7 samples/market/week) were collected by convenience sampling. Real-time RT-PCR [19] and viral cultures were performed on swine specimens at UMN. Real-time RT-PCR samples with Ct values <35 were considered positive, between 35 and <40 were suspect, and >40 were negative. Samples with Ct values <40 were selected for culture in MDCK cells [20]. Whole-genome sequencing (WGS), phylogenetic analysis, and dynamics of genetic variation analysis of viral isolates were done as described in the Supplementary Methods.

Environmental Surveillance

Once a week, air samples were collected from the animal holding and the animal processing areas by using a cyclonic air

collector [12] (Midwest Micro-Tek, Brookings, South Dakota) capable of sampling 200 L of air/minute. Air samples were collected in the mornings at approximately the same time of day and mostly on the same day of the week. Air collectors were placed inside swine pens approximately 1–1.5 meters above the ground and operated for approximately 20 minutes.

Surface samples were taken from areas considered of high contact by humans, which included swine pen railings, sink handles, and faucets inside the customer area, and doorknobs leading into the animal holding area. Surface samples were collected by using disposable gloves and gauzes dipped into sterile minimum essential medium with 2% bovine serum albumin. One-meter sections of pen railing were wiped for 30 seconds. Sink handles, faucets, and exterior and interior door handles were wiped for 10 seconds. Environmental testing, including quantitative rRT-PCR [10] and viral culture, were performed at UMN. WGS, phylogenetic analysis, and dynamics of genetic variation analysis of viral isolates were done as described in the Supplementary Methods.

RESULTS

Illness Surveillance Among Employees

Seventeen of 22 employees (4 from market A and 13 from market B) participated in this study. Of these, 16 employees were Hmong and one was Hispanic. No employee reported medical comorbidities. At market A, employees (n = 4) performed both swine care and butchering duties. Market B employees worked as butchers (n = 10), clerical support personnel (n = 2), or swine care attendant (n = 1). Employees at both markets reported wearing rubber boots and rubber gloves the majority of the time, but they did not wear face masks.

One hundred sixty-five nasal swabs were collected. Twenty-two samples, representing 11 of 17 (65%) employees, were positive for IAVs (Figure 1). Of these, only 1 employee reported illness (fever, headache, or rhinorrhea) the week preceding sample collection. Seven employees tested positive for IAVs by rRT-PCR on multiple occasions, including 1 employee in whom seasonal and variant IAVs were identified at different times (Supplementary Table 1). IAVs detected included variant H3 (n = 4) and seasonal H3 (n = 7) viruses. Eleven nasal swabs positive for IAV were not subtyped because of insufficient virus quantity (Ct 32–37). Viral cultures of all IAV rRT-PCR–positive nasal specimens (Ct 32–37) yielded no growth.

Among the 15 employees whose job duties involved direct swine contact, 11 (73%) had baseline HI antibody titers ≥40 to both swine H1N2 IAVs tested; 10 (67%) had baseline ≥40 HI titers to A/Ohio/13/2012, and 6 (40%) had baseline ≥40 HI titers to A/Mexico/4108/2009 virus. One employee, a butcher employed at market B for 2 months, had 4-fold increases in both HI and MN antibody titers to 2 swine H1N2
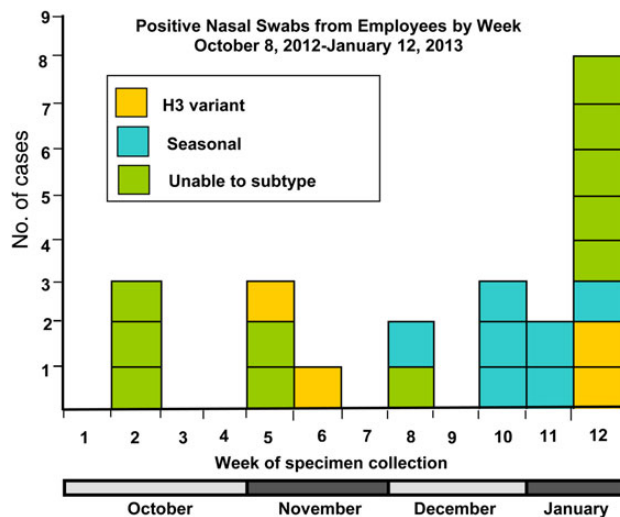
and A/Mexico/4108/2009 viruses (Supplementary Table 2). He tested positive for seasonal H3 virus during week 10 and for variant H3 at week 12 by rRT-PCR. He was not vaccinated for seasonal influenza during the surveillance period and never reported experiencing influenza-like illness.

Swine and Environmental Surveillance

Real-time RT-PCR was performed on 364 swine and environmental samples (Table 1). IAVs were detected in swine lungs (70/150), oral fluids (47/49), air samples from animal holding areas (30/57), swine pen railings (16/34), door leading to the animal holding area (1/25), and a sink or faucet located inside the customer area (1/24). No IAVs were detected in air samples from the animal processing area (0/25). IAVs were cultured from swine lungs (72/84), oral fluids (13/46), air samples from the animal holding area (30/45), swine pen railings (5/21), door to the animal holding area (1/4), and a sink or faucet located inside the customer area (2/4).

One hundred twenty-two IAV isolates underwent WGS (1 isolate could not be sequenced). These isolates included subtypes H1N1 (n = 3), H1N2 (n = 32), H3N2 (n = 78), and multiple subtype codetections (n = 9) (Table 1). H1N1 IAVs were isolated only from swine lung specimens. H1N2 IAVs were isolated from swine lungs, oral fluids, and air samples, whereas H3N2 viruses were isolated from all sample types tested. Mixtures of IAV subtypes were cultured from swine lungs, oral fluids, and air samples, including H1N2 and H3N2, H1N1 and H3N2, and H3N2s of 2 different lineages (Figure 2; Supplementary Table 3). Genetically similar IAV isolates were identified in samples from swine and environmental specimens in both markets (Supplementary Figure 1).

Figure 1. Positive influenza A virus real-time reverse transcription polymerase chain reaction nasal swabs from employees, by week.

Table 1. Number of Influenza A Viruses Identified by Real-time Reverse Transcription Polymerase Chain Reaction and Virus Isolation by Sample Type, and Number of Viral Isolates by Subtype for the Swine and Environmental Surveillance

Isolate	No. IAV rRT-PCR Positive/No. Tested (%)	No. IAV Isolation Positive/No. Tested[a] (%)	Subtype[b] (No. of Virus Isolates)
Swine lungs	70/150 (47)	72/84 (86)	H1N1 (n = 3), H1N2 (n = 22), H3N2 (n = 39), codetections (n = 7)
Oral fluids	47/49 (96)	13/46 (28)	H1N2 (n = 3), H3N2 (n = 9), codetections (n = 1)
Air, swine pens	30/57 (53)	30/45 (67)	H1N2 (n = 7), H3N2 (n = 22), codetections (n = 1)
Air, processing area	0/25 (0)	NT	NT
Railings, swine pens	16/34 (47)	5/21 (24)	H3N2 (n = 5)
Door, animal holding area	1/25 (4)	1/4 (25)	H3N2 (n = 1)
Sink or faucet	1/24 (4)	2/4 (50)	H3N2 (n = 2)
Total	164/364 (45)	123/204 (60)	H1N1 (n = 3), H1N2 (n = 32), H3N2 (n = 78), codetections (n = 9)

Abbreviations: IAV, influenza A virus; NT, not tested; rRT-PCR, real-time reverse transcription polymerase chain reaction.

[a] No. tested for virus isolation included rRT-PCR positive (cycle threshold <35) and suspect (cycle threshold 35–40) samples.

[b] One isolate could not be subtyped or sequenced.

IAV isolates from swine and environmental specimens were genetically diverse and belonged primarily to 3 lineages (triple-reassortant internal gene, classic swine, and H1N1pdm09) of IAVs known to circulate among commercial swine in North America (Supplementary Table 3). Substantial genetic variation among IAV isolates among and within subtypes, with multiple distinct genome constellations were identified. Eighty-five percent (69/81) of H3N2 isolates had a similar genome constellation to the one identified in human H3N2v cases [21]. Each market carried distinct IAV gene constellations defined by different genetic patterns and genome dynamics over time (Figure 2; Supplementary Figure 2). Differences in reassortment dynamics for each location were reported. Market A had greater diversity among IAV isolates, and genetic constellations were more dynamic than in market B (Figure 2). Multiple gene segment exchange events among and within subtypes were noted, which in certain instances, led to new constellations of gene segments and the identification of new viruses. New gene segments were detected that appeared to transmit efficiently among swine within limited time after introduction (Figure 2). A new gene segment was defined as a gene segment that had not been identified among IAV isolates sequenced during preceding sampling events for that market.

Market Customer With Confirmed H3N2v Infection

On 7 December 2012, a boy aged 12 years with H3N2v IAV infection (rRT-PCR at MDH) was identified through routine influenza surveillance [9]. On 9 November 2012, 3 days before illness onset, the boy had gone to market B. During that visit, he entered the swine barn and touched swine pen railings and a live swine. He reported no other swine exposure. The H3N2v virus sequence recovered from the child was 99%–100% similar to 7 gene segments from a H3N2 virus isolated from a swine lung specimen collected on 1 November 2012.

Segment 8 (non-structural gene) from the male was 100% similar to that of 3 swine H1N2 viruses recovered from swine lung specimens collected on 8 November 2012.

DISCUSSION

This is the first comprehensive characterization of IAVs among live animal markets in the United States that incorporated surveillance of persons, swine, and the environment. IAVs of swine origin were highly prevalent among swine and were readily isolated from environmental samples, especially air, providing evidence that air might be an important route of IAV transmission. We report that multiple IAV strains and subtypes were co-circulating, identified new viral reassortants, and provided evidence indicating interspecies transmission of IAV from swine to persons.

Swine tested positive for IAVs throughout the study period at both markets. Viable IAVs were isolated from more than half of the air samples, providing evidence that air can be an important route of IAV transmission among swine and from swine to persons. H3N2 was the IAV subtype isolated more frequently from swine and environmental samples, although codetections with multiple subtypes were common. The majority of H3N2 isolates had a genome constellation previously identified among human H3N2v viruses [21]. Whether this represents a higher prevalence of H3N2 subtype or better adaptability to cell culture is unclear, but it might indicate a greater risk for transmissibility. Overall, we hypothesize that living virus encountered in the environment represents a risk to persons attending the markets.

We isolated a mixture of genetically diverse IAVs from swine and environmental samples belonging to 3 different swine HA genetic lineages and identified multiple gene segment exchanges among and within IAV subtypes. Although differences in reassortment dynamics between markets were observed, in certain

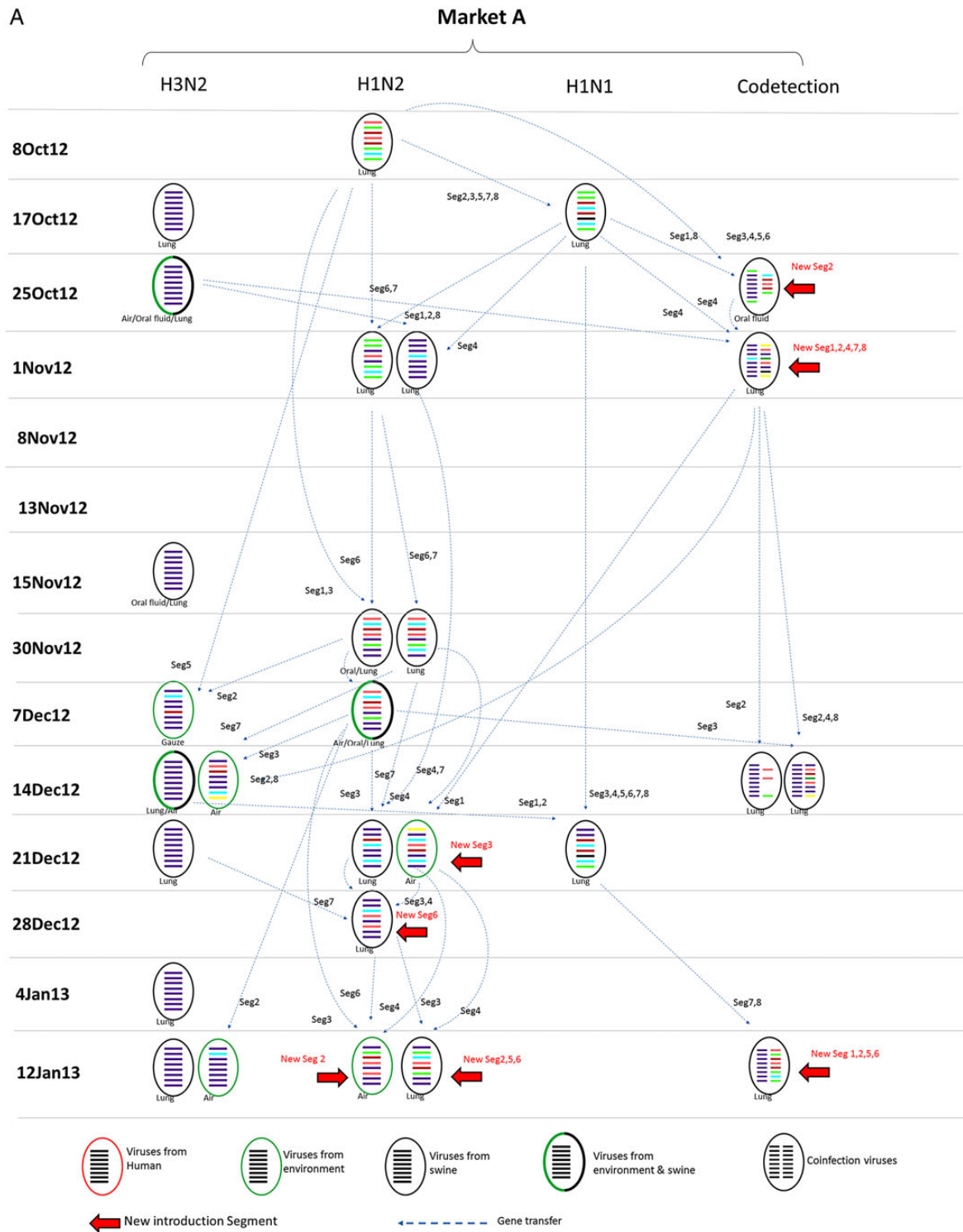**Figure 2.** Diagram of genetic diversity and gene segment relatedness of influenza A virus (IAV) isolates recovered from swine and environmental specimens from market A and market B, including the live animal market visitor who received a diagnosis of H3N2 variant infection. Dynamics of genetic constellations and reassortment events were assessed by using Bayesian Markov chain Monte Carlo inference framework implemented in Bayesian evolutionary analysis by sampling trees. Gene color segments represent the group where they cluster in the phylogenic tree based on posterior probability of >0.75 (Supplementary Figure 2). Blue arrows indicate that samples in the next period contained the same gene segments from the prior period. Red arrows indicate that a new gene segment was detected for the first time in the study for that facility. Oval colors represent IAVs isolated from market visitor (red), environmental specimens (green), swine (black), and environmental specimens and swine (green and black). Ovals with 2 sets of gene segments indicate codetection with different IAV subtypes in the same specimen.
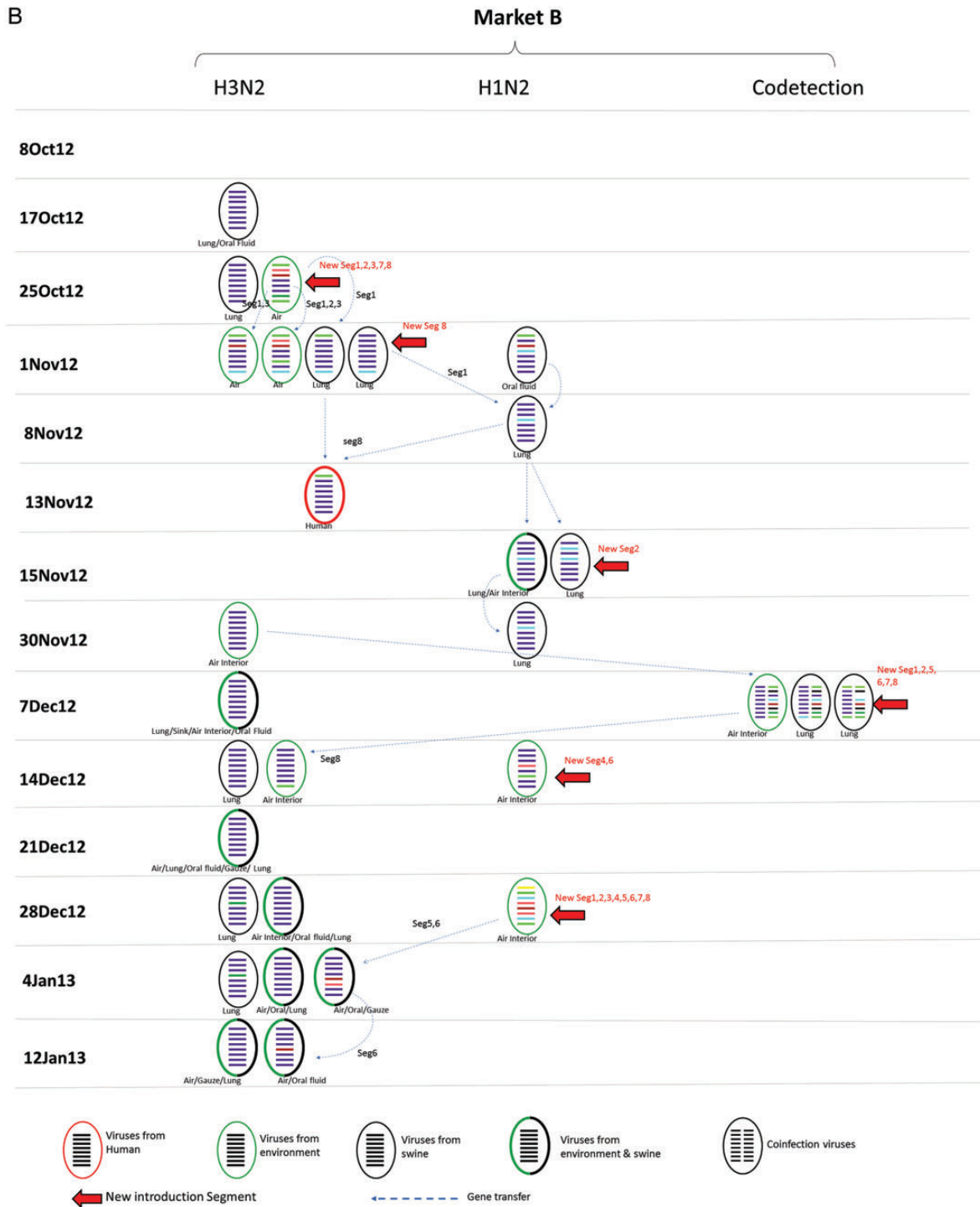
Figure 2 continued.

instances, these gene exchange events led to new genome constellations and identification of new viruses. Although a common source strain(s) of the market isolates could serve as a donor for any specific segment to generate novel reassortants, our sampling protocols and massive parallel genome sequencing suggest that most new reassortant constellations were generated from strains identified in a temporally predictable fashion in the markets. In part, the dynamic genetic environment observed might be from unique swine-handling practices at live animal markets (eg, purchasing swine from multiple sources, holding swine of varying ages together, and holding swine from week to week). Also, repeated interactions among market employees and

customers of all ages with swine make live animal markets a venue where swine can be exposed to human IAVs. This is an important consideration because only 1 study participant had been vaccinated against seasonal influenza.

Of the 11 employees with IAV-positive respiratory samples, only 1 reported illness during the week preceding sample collection. Whether IAV-positive specimens collected from asymptomatic employees represented subclinical infections or transient nasal deposition of IAVs is unknown. Short-term mechanical carriage of IAV in nostrils has been documented among persons involved with H5N2 avian IAV outbreaks [22]. Furthermore, because the amount of viral RNA detected by rRT-PCR was low, the diversity observed among IAV subtypes was potentially an artifact of Ct values approaching the limit of detection. However, the abundance and diversity of IAVs detected in air samples demonstrates that employees were frequently being exposed to a variety of IAVs present in the air. Approximately two-thirds of employees had HI and MN antibody titers >40 to H3N2v (A/Ohio/13/2012) and 2 swine H1N2 viruses at baseline. Although the possibility exists that these titers represent cross-reactive antibodies because of exposure to human IAVs [23] or past exposure to seasonal H3N2 viruses, particularly viruses that circulated during the 1990s [24], we cannot rule out the possibility that these antibodies were result of past exposure to swine IAVs. Overall prevalence of HI titers ≥40 against H3N2v was higher than the one reported for the general population [23].

A market customer received a diagnosis of variant IAV infection during the study period. When sequenced, the virus isolated from his respiratory specimen contained gene segments that were >99% similar to segments identified in 4 distinct IAVs circulating among swine at market B during the week preceding and the week of his market visit. Although we did not find the complete 8-gene combination in a single isolate, this is a reflection of the dynamic exchange of genetic material among IAVs occurring at the markets. It is unlikely that this same viral strain circulated, unnoticed, in communities such as schools in Minnesota. These findings support swine-to-human transmission of IAVs at the markets, and are consistent with other studies where live bird markets have been implicated in the transmission of H7N9 and H5N1 IAVs from poultry to persons [10, 11, 25, 26].

This study highlights the importance of a multidisciplinary approach to novel IAV surveillance and response involving state and federal agencies of public health and agriculture, academia, and private industry. Our findings raise concern that conditions at live animal markets might facilitate emergence of novel IAVs. If efficiently transmitted among humans, novel IAVs might have pandemic potential, prompting the need for public health and agriculture agencies to work with community groups, market employees, and market customers to develop culturally appropriate messaging about the risk for variant IAV infections in these settings. Encouraging seasonal flu vaccination, educating customers about prevention measures against IAV, and promoting the use of PPE among employees is also important.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

References

1. Centers for Disease Control and Prevention. Variant (swine origin) influenza viruses in humans. Available at: http://www.cdc.gov/flu/swineflu/variant.htm. Accessed 2 September 2014.
2. Jhung MA, Epperson S, Biggerstaff M, et al. Outbreak of variant influenza A(H3N2) virus in the United States. Clin Infect Dis 2013; 57:1703–12.
3. Wong KK, Greenbaum A, Moll ME, et al. Outbreak of influenza A (H3N2) variant virus infection among attendees of an agricultural fair, Pennsylvania, USA, 2011. Emerg Infect Dis 2012; 18:1937–44.
4. Wells DL, Hopfensperger DJ, Arden NH, et al. Swine influenza virus infections. Transmission from ill swine to humans at a Wisconsin

agricultural fair and subsequent probable person-to-person transmission. JAMA **1991**; 265:478–81.

5. Yassine HM, Khatri M, Zhang YJ, et al. Characterization of triple reassortant H1N1 influenza A viruses from swine in Ohio. Vet Microbiol **2009**; 139:132–9.

6. Cox CM, Neises D, Garten RJ, et al. Swine influenza virus A (H3N2) infection in human, Kansas, USA, 2009. Emerg Infect Dis **2011**; 17:1143–4.

7. Dawood FS, Dong L, Liu F, et al. A pre-pandemic outbreak of triple-reassortant swine influenza virus infection among university students, South Dakota, 2008. J Infect Dis **2011**; 204:1165–71.

8. Bowman AS, Sreevatsan S, Killian ML, et al. Molecular evidence for interspecies transmission of H3N2pM/H3N2v influenza A viruses at an Ohio agricultural fair, July 2012. Emerg Microbes Infect **2012** 1:e33.

9. Choi MJ, Morin CA, Scheftel J, et al. Variant influenza associated with live animal markets, Minnesota. Zoonoses Public Health **2015**; 62:326–30.

10. Wang C, Wang J, Su W, et al. Relationship between domestic and wild birds in live poultry market and a novel human H7N9 virus in China. J Infect Dis **2014**; 209:34–7.

11. Wan XF, Dong L, Lan Y, et al. Indications that live poultry markets are a major source of human H5N1 influenza virus infection in China. J Virol **2011**; 85:13432–8.

12. Corzo CA, Culhane M, Dee S, Morrison RB, Torremorell M. Airborne detection and quantification of swine influenza a virus in air samples collected inside, outside and downwind from swine barns. PLoS One **2013**; 8:e71444.

13. Allerson MW, Cardona CJ, Torremorell M. Indirect transmission of influenza A virus between pig populations under two different biosecurity settings. PLoS One **2013**; 8:e67293.

14. Ito T, Couceiro JN, Kelm S, et al. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. J Virol **1998**; 72:7367–73.

15. Shu B, Wu K-H, Emery S, et al. Design and performance of the CDC real-time reverse transcriptase PCR swine flu panel for detection of 2009 A (H1N1) pandemic influenza virus. J Clin Microbiol **2011**; 49:2614–9.

16. World Health Organization. CDC protocol of real-time RT-PCR for swine influenza A (H1N1). Geneva, Switzerland: WHO, **2009**. Available at: http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20090428.pdf. Accessed 2 September 2014.

17. World Health Organization. Manual for the laboratory diagnosis and virological surveillance of influenza. Geneva, Switzerland: WHO, **2011**, pp 59–78. Available at: http://www.who.int/influenza/gisrs_laboratory/manual_diagnosis_surveillance_influenza/en/. Accessed 2 September 2014.

18. Romagosa A, Gramer M, Joo HS, Torremorell M. Sensitivity of oral fluids for detecting influenza A virus in populations of vaccinated and non-vaccinated pigs. Influenza Other Respir Viruses **2012**; 6:110–8.

19. Slomka MJ, Densham AL, Coward VJ, et al. Real time reverse transcription (RRT)-polymerase chain reaction (PCR) methods for detection of pandemic (H1N1) 2009 influenza virus and European swine influenza A virus infections in pigs. Influenza Other Respir Viruses **2010**; 4:277–93.

20. Meguro H, Bryant JD, Torrence AE, Wright PF. Canine kidney cell line for isolation of respiratory virus. J Clin Microbiol **1979**; 9:175–9.

21. Kitikoon P, Nelson MI, Killian ML, et al. Genotype patterns of contemporary reassorted H3N2 virus in US swine. J Gen Virol **2013**; 94: 1236–41.

22. Bean WJ, Kawaoka Y, Wood JM, Pearson JE, Webster RG. Characterization of virulent and avirulent A/chicken/Pennsylvania/83 influenza A viruses: potential role of defective interfering RNAs in nature. J Virol **1985**; 54:151–60.

23. Skowronski DM, Janjua NZ, De Serres G, et al. Cross-reactive and vaccine-induced antibody to an emerging swine-origin variant of influenza A virus subtype H3N2 (H3N2v). J Infect Dis **2012**; 206:1852–61.

24. Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science **2009**; 325:197–201.

25. Bao CJ, Cui LB, Zhou MH, et al. Live-animal markets and influenza A(H7N9) virus infection. N Engl J Med **2013**; 368:2337–9.

26. Wang M, Di B, Zhou DH, et al. Food markets with live birds as source of avian influenza. Emerg Infect Dis **2006**; 12:1773–5.