

Relationship Between Domestic and Wild Birds in Live Poultry Market and a Novel Human H7N9 Virus in China

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To trace the source of the avian H7N9 viruses, we collected 99 samples from 4 live poultry markets and the family farms of 3 patients in Hangzhou city of Zhejiang province, China. We found that almost all positive samples came from chickens and ducks in live poultry markets. These results strongly suggest that the live poultry markets are the major source of recent human infections with H7N9 in Hangzhou city, Zhejiang province of China. Therefore, control measures are needed, not only in the domestic bird population, but also in the live poultry markets to reduce human H7N9 infection risk.

Keywords. live poultry market; domestic avian; wild bird; human H7N9; China.

Outbreaks of low-pathogenicity avian influenza A viruses (H7N2, H7N3, H7N7, H9N2, and H10N7) in humans have been reported in different areas of the world [1]. However, sporadic human infections with the H7N9 avian influenza virus had never been documented anywhere in the world until March 2013 when the National Health and Family Planning Commission of China (NHFPC) reported the first human infections caused by a novel avian-origin influenza A (H7N9) virus [2]. The first 3 cases of human infection with this novel

H7N9 virus in Shanghai and Anhui Province were virulent and fatal. As of 31 May 2013, the National Health and Family Planning Commission of China (NHFPC) had reported 131 confirmed human infections and 39 deaths from the H7N9 virus in 9 provinces: Shanghai, Jiangsu, Anhui, Beijing, Zhejiang, Fujian, Jiangxi, Henan, and Taipei, which has raised concerns about the potential of this novel virus to cause a future influenza pandemic.

Novel reassortant avian-origin influenza A (H7N9) viruses were recently isolated from 3 patients in China [2]. Sequence analyses of the viral genomes revealed that 6 gene segments encoding internal proteins were from avian influenza virus A/brambling/Beijing/16/2012 (H9N2), and 2 gene segments encoding surface proteins were derived from avian influenza viruses A/duck/Zhejiang/12/2011 (H7N3) and A/wild bird/Korea/A14/2011 [2]. Although it has not caused any outbreaks owing to its low pathogenicity in chickens and ducks, the reassortant avian H7N9 virus may have circulated in domestic poultry (eg, chickens and ducks) before the first human infections were discovered in March 2013. Historically, H7 influenza A viruses have resulted in >100 cases of human infection since 2002 in the Netherlands, Italy, Canada, the United States, China, and the United Kingdom [1, 2, 3–7]. Live poultry markets were reported to act as reservoirs of the H5N1 virus despite fewer reported outbreaks on poultry farms [8, 9]. The major aim of this study was to trace the source of this novel reassortant avian-origin influenza A (H7N9) virus, which caused human infections, by investigating live poultry markets in Hangzhou, China.

METHODS

Sample Collection From Live Poultry Markets

Ninety-five throat swab, anal swab, and fecal samples were obtained from chickens, ducks, quails, pigeons, and poultry sales staff in 4 live poultry markets and at the family farms of 3 patients infected by H7N9 virus in Hangzhou city of Zhejiang province from April 4 through 12. Four water samples were also collected in or near the live poultry markets.

Detection and Identification of Samples

The collected specimens were propagated in the allantoic sac and amniotic cavity of specific pathogen-free embryonated chicken eggs (9–11 days old) for 48–72 hours at 35°C and were analyzed by using a subtype-specific real-time reverse-transcriptase polymerase chain reaction assay [2].

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RESULTS

Our results showed that H7N9 viral RNAs were detected in 48% of all collected samples (41 of 85), 40% of the chicken samples (19 of 47), 89% of the duck samples (8 of 9), and 33% of the pigeon samples (1 of 3) (Table 1). Furthermore, the detection rate for H7N9 viral RNAs was as high as 66% (41 of 62) in samples collected from 4 live poultry markets, and almost all of these positive samples came from chickens and ducks. In addition, all of the environmental samples, especially 4 samples (100%) of waste water from the sewerage or processing of live birds in live poultry markets, were found to be positive for H7N9 viral RNAs, suggesting that they may play an important role in the emergence and spread of this novel avian H7N9 virus among chickens and ducks in live poultry markets and in the transmission of this virus to humans. This finding has important biological significance for effective prevention and control of human infections by this novel avian-origin H7N9 influenza virus. Surprisingly, none of the 37 samples collected from the family farms of 3 patients or the 14 serum or swab samples collected from employees working in live poultry markets were found to be positive for H7N9 viral RNA

(Table 1). It is not clear why these individuals who were working in live poultry markets with routine exposure to chickens or ducks carrying the H7N9 virus were not infected, unlike some others who came to the markets occasionally.

DISCUSSION

Other study revealed that H7N9 human influenza viruses in China are naturally occurring reassortant viruses from domestic or wild birds. Phylogenetic analysis confirmed that 2 of the genome segments from the reassortant viruses (HA and NA) originated from 4 ancestral duck and wild bird viruses, and the remaining 6 segments (PB2, PA, M, NP, PB1, NS) descended from 9 ancestral brambling, chicken, duck, and pigeon viruses [10]. Although the reassortant viruses are human isolates, many associated avian infections in live poultry markets are epidemiologically linked (Table 1), suggesting zoonotic transmission from domestic birds. The underlying uncertainty in estimated phylogenies means that we cannot rule out the possibility of a single origin. However, it is a fact that the outbreak of H7N9 avian influenza in humans has a close relationship with domestic and wild birds.

Table 1. Detection of H7N9 Virus RNA in Specimens Collected From 4 Live Poultry Markets and 3 Family Farms in Hangzhou City, Zhejiang Province, China

| Samples | Samples Tested, No. | | | | | | | |
|---------------------------------------|----------------------|-------------|-----------|------------|---------------------------------|-----------|-----------|------------|
| | Live Poultry Markets | | | | Family Farms | | | |
| | Dongxin | Wuhang | Binsheng | Zhanongkou | Patient 1 | Patient 2 | Patient 3 | Total |
| Chicken | | | | | | | | |
| Swab | 0 | 10 | 4 | 0 | 14 (7 serum and 7 swab samples) | 0 | 0 | 10/28 (36) |
| Feces | 4 | 0 | 1 | 2 | 7 | 3 | 0 | 7/17 (41) |
| Feather smear | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2/2 (100) |
| Subtotal ^a | 4/4 (100) | 12/12 (100) | 1/5 (20) | 2/2 (100) | 0/21 | 0/3 | 0 | 19/47 (40) |
| Duck | | | | | | | | |
| Swab | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 4/5 (80) |
| Feces | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3/3 (100) |
| Feather smear | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1/1 (100) |
| Subtotal ^a | 3/3 (100) | 5/5 (100) | 0/1 | 0 | 0 | 0 | 0 | 8/9 (89) |
| Quail | | | | | | | | |
| Swab | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0/1 |
| Feces | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/0 |
| Feather smear | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0/1 |
| Subtotal ^a | 0 | 0 | 0/2 | 0 | 0 | 0 | 0 | 0/2 |
| Pigeon^a | | | | | | | | |
| Streak-throated fulvetta ^a | 0 | 0 | 1/1 (100) | 0 | 0 | 0/2 | 0 | 1/3 (33) |
| Environment ^a | 1/1 (100) | 7/7 (100) | 1/1 (100) | 0 | 0/7 | 0 | 0 | 9/16 (56) |
| Water ^a | 0 | 1/1 (100) | 2/2 (100) | 1/1 (100) | 0 | 0 | 0 | 4/4 (100) |
| Total ^a | 8/8 (100) | 25/25 (100) | 5/12 (42) | 3/3 (100) | 0/28 | 0/5 | 0/4 | 41/85 (48) |

Throat and anal swabs were obtained from chicken, duck, quail, and pigeon samples. Only throat swabs were obtained from human patients.

^a Data represent No. of H7N9-positive samples/No. of samples tested (%).

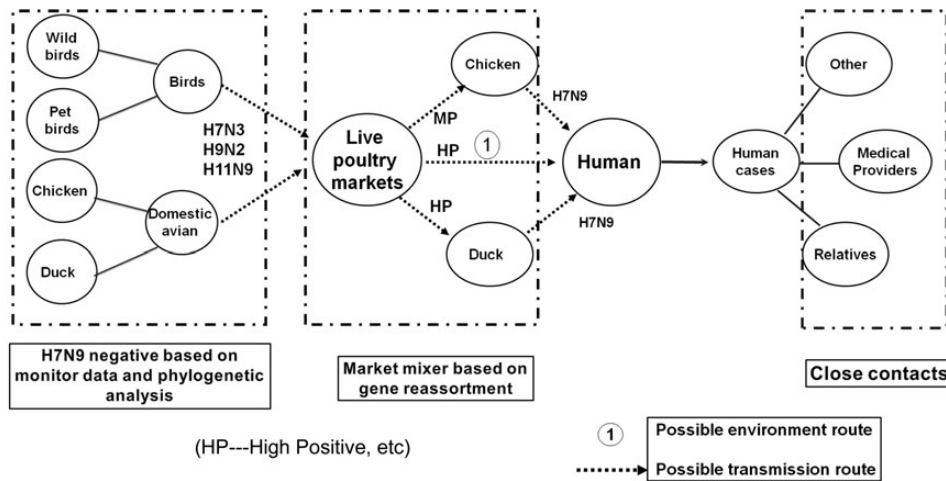


Figure 1. Proposed hypothesis that the live poultry market is the place where the novel H7N9 viruses emerged recently and acquired the capability of infecting humans directly through reassortment among different influenza viruses from birds and domestic chickens or ducks as well as accumulation of new mutations in important positions of different viral genes. Abbreviations: HP, high positive; MP, medium positive.

Live food markets have long been suspected of being reservoirs for the deadly H5N1 avian flu; Wan et al [8] tested 69 environmental samples from the cages, floors, and ditches at 6 wet markets during the 2008–2009 flu season and found H5N1 at 4 of the markets. The present study shows that 85 samples collected from poultry or water samples from poultry markets, 40% of the chicken samples, 89% of the duck samples, and 33% of the pigeon samples were positive for H7N9 influenza viruses. Based on these samples, chicken, pigeon and duck were found to be the major hosts of H7N9. In addition, the positive rate for environmental samples (especially waste water) in live poultry markets reached 100%. These studies are more in keeping with the results of a small number of samples reported in the another investigation, in which 1 of 13 samples from a poultry market were positive [11]. Another study also demonstrated high levels of HPAI A(H5N1) virus contamination in live poultry markets in Cambodia [12]. However, the reports from the Ministry of Agriculture showed that testing of tens of thousands of samples from poultry and their environment has identified 51 H7N9 virus isolates from the provinces of Anhui, Henan, Zhejiang, Fujian, and Jiangsu, as well as the Shanghai municipality, mostly from live poultry markets, showing a low positive rate (.005%, 51 of 899 758 sample) [13–15].

We think that 2 alternative possibilities coexist. The first is the difference in sample sites. As we know, the selected sites for sample collection during the large-scale investigation focused on migratory bird routes, live poultry markets, wet lands, poultry farms, swine farms, and so on. However, our study focused mainly on the live poultry markets, where many patients, including 3 patients in our study, have had close contact and near to which there is a larger infected population in Hangzhou city. Another possibility is that the numbers of samples from each

species and from the environment are somewhat limited in our study. Another interesting finding is that samples from the family farms of the 3 patients and employees were shown to be negative for H7N9 virus RNA; the reason for this needs further investigation.

These findings are important because they reveal the need for improved veterinary monitoring and control in the wet markets as well as faster culling when infections are identified. H7N9 is still primarily passed directly to humans through infected animals and has a high mortality rate among humans. However, because the live poultry markets facilitate the comingling of numerous species, including fowl and wild birds, they are a likely breeding ground for deadly genetic reassortments that could make the virus easily transmissible between humans. Live markets may play an important role in the emergence and spread of this novel avian H7N9 virus among chickens and ducks and the transmission of this virus to humans.

Migratory birds seem to serve as the reservoir for most influenza A viruses. Although humans do not seem to be directly infected by H7N9 viruses from wild birds, domestic birds can support the replication of both human and wild bird viruses. Coinfection of domestic birds by human and wild bird viruses is thought to favor genetic reassortment, which may generate hybrid viruses capable of infecting humans. This relationship between human influenza pandemics and wild bird reservoirs of the influenza A virus has stimulated considerable interest in the virus strains that infect these birds and the mechanisms responsible for their maintenance.

Bramblings, a migratory species that winters in southern China and is widespread throughout the forests of China (Supplementary Figure 1), is suggested to be the reservoir of H7N9 because reassortant virus strains are produced in multiple

subtype virus co-infected cells in birds or other animals (although H7N9 is not isolated from this bird now). They also winter in southern Europe, northern Africa, northern India, northern Pakistan, and Japan, and they regularly stray into Alaska during migration and may continue as far south as the western United States. All of the human cases have occurred along the migratory route of these wild birds. Thus, wild birds carrying well-adapted H7N9 influenza A viruses may provide a possible route for the reassortant of avian viruses with human and other mammalian viruses during the annual migration of the wild birds from Asia to other regions, including North America. Efforts to control the Baikal teal and brambling will be important in the future.

Gao et al [2] demonstrated that novel reassortant avian-origin influenza A (H7N9) viruses are associated with rapidly progressing and fatal lower respiratory tract infections in 3 patients in China. However, the source of reassortant H7N9 viruses and the mode of their transmission to humans remain unclear. Based on our current findings that a high percentage of samples collected from live poultry markets but none collected from poultry farms were positive for H7N9 viral RNA, we propose a hypothesis (Figure 1) that the live poultry market is the place where the novel H7N9 viruses emerged recently and acquired the ability to infect humans directly through reassortment among different influenza viruses from birds and domestic chickens or ducks as well as accumulation of new mutations in important positions of different viral genes. This hypothesis can help us implement measures to effectively control the spread of these viruses to humans.

In conclusion, our results strongly suggest that the live poultry markets are the major source of the recent human infections with the novel avian H7N9 viruses in Hangzhou city, Zhejiang province, China. Human infections with the novel H7N9 viruses were originally detected in approximately March 2013 in 11 provinces of China, and the virus was spread to other provinces or regions of China, as demonstrated by the detection of subtypes of the H7N9 influenza virus in humans in Beijing and Henan since 13 April 2013. The future spread of the H7N9 viruses in humans will probably continue to occur with the northward movement of migratory birds. Finding the source of novel H7N9 viruses and the routes by which humans were infected with them and understanding their pandemic potential will further enhance our ability to respond to and reduce the severity of future pandemics. Therefore, control measures are urgently needed, not only in the domestic bird populations, but also in the live poultry markets to reduce the risk of human infections with novel H7N9 viruses.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.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

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Wang CM, Luo J, Wang J, et al. Novel human H7N9 influenza virus in China. *Integ Zool* **2013**; 8:327–329. doi:10.1111/1749-4877.12047.
2. Gao R, Cao B, Hu Y, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. *N Engl J Med* **2013**; 368:1888–97.
3. Tweed SA, Skowronski DM, David ST, et al. Human illness from avian influenza H7N3, British Columbia. *Emerg Infect Dis* **2004**; 10:2196–9.
4. Thompson WW, Shay DK, Weintraub E, et al. Influenza-associated hospitalizations in the United States. *JAMA* **2004**; 292:1333–40.
5. Skowronski DM, Li Y, Tweed SA, 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.
6. Kuhne M, Morgan O, Ellis J, et al. Human antibody response to avian influenza A (H7N3) virus during an outbreak in poultry in Norfolk, United Kingdom. *Options for the Control of Influenza VI*, 17–23 June 2007, Toronto, Ontario, Canada.
7. Avian influenza A/(H7N2) outbreak in the United Kingdom. *Euro Surveill* **2007**; 12:E070531.2.
8. Wan XF, Dong LB, 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.
9. Jessica A, Belsler CB, Bridges JM, Katz TM. Tumpey, past, present, and possible future human infection with influenza virus A subtype H7. *Emerg Infect Dis* **2009**; 15:859–65.
10. Liu D, Shi WF, Shi Y, et al. Origin and diversity of novel avian influenza A H7N9 viruses causing human infection: phylogenetic, structural, and coalescent analyses. *Lancet* **2013**; 381:1926–32.
11. Bao CJ, Cui LB, Zhou MH, Hong L, Gao GF, Wang H. Live-animal markets and influenza A (H7N9) virus infection. *N Engl J Med* **2013**; 368:2337–9.
12. Srey VH, San S, Lotfi A, Philippe B. Influenza A(H5N1) virus surveillance at live poultry markets, Cambodia, 2011. *Emerg Infect Dis* **2013**; 19:305–8.
13. World Health Organization. Overview of the emergence and characteristics of the avian influenza A (H7N9) virus. **2013**. http://www.who.int/influenza/human_animal_interface/influenza_h7n9/WHO_H7N9_review_31May13.pdf. Accessed 31 May 2013.
14. Office of International Epizootics (OIE). Low pathogenic avian influenza (poultry) China (People's Rep. of). Follow-up report No. 6. **2013**. http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=13314. Accessed 16 May 2013.
15. Office of International Epizootics (OIE). Low pathogenic avian influenza (poultry) China (People's Rep. of). Immediate notification. **2013**. http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=13225. Accessed 16 May 2013.