

Table. Prevalence of *Bartonella* spp. in bats from 2 sites in Dong Nai, Vietnam, 2013

Bat species	No. <i>Bartonella</i> spp.–positive bats/no. bats trapped (%)		
	Cat Tien National Park	Dong Nai Nature Reserve	Total
<i>Cynopterus sphinx</i> *	0/0	0/14	0/14 (0)
<i>Hipposideros armiger</i> †	2/6	0/0	2/6 (33.3)
<i>Hipposideros larvatus</i> †	3/5	0/0	3/5 (60)
<i>Megaerops niphanae</i> *	0/0	1/2	1/2 (50)
<i>Megaderma spasma</i> †	0/0	1/2	1/2 (50)
<i>Megaderma lyra</i> ‡	1/1	0/0	1/1 (100)
<i>Rhinolophus acuminatus</i> †	0/0	9/17	9/17 (52.9)
<i>Rhinolophus chaseli</i> †	2/5	0/0	2/5 (40)
<i>Rhinolophus sinicus</i> †	0/3	2/4	2/7 (28.6)
<i>Rhinolophus luctus</i> †	0/1	0/0	0/1 (0)
Total	8/21 (38.1)	13/39 (33.3)	21/60 (35)

*Fruit-eating.

†Insectivorous.

‡Carnivorous.

caused by crowded roosting areas and sharing of roosts by multiple species. This behavior provides opportunities for transmission of *Bartonella* bacteria or exchange of infected ectoparasites, such as *Cyclopodia* spp. (8), although the precise roles of these 2 processes are unknown.

Although no human cases of *Bartonella* spp. infection have been reported in Vietnam, *Bartonella* spp. have been identified in febrile humans elsewhere in Southeast Asia (9) and are also common in rats in southern Vietnam (10). Because close contact with bats (i.e., through manure farming and consumption of bat meat) and potential arthropod vectors (i.e., through handling and consumption of fruit) is common in parts of Vietnam, targeted screening of bats and their human contacts might improve our understanding of the zoonotic potential of these bacteria and their potential effect on public health.

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References

- Kruskop SV. Bats of Vietnam. Checklist and an identification manual. Moscow: KMK Scientific Press; 2013.
- Francis CM. A guide to the mammals of Southeast Asia. Princeton (NJ): Princeton University Press; 2008.
- Billeter SA, Hayman DT, Peel AJ, Baker K, Wood JL, Cunningham A, et al. *Bartonella* species in bat flies (Diptera: Nycteribiidae) from western Africa. *Parasitology*. 2012;139:324–9. <http://dx.doi.org/10.1017/S0031182011002113>
- La Scola B, Zeaiter Z, Khamis A, Raoult D. Gene-sequence-based criteria for species definition in bacteriology: the *Bartonella* paradigm. *Trends Microbiol*. 2003;11:318–21. [http://dx.doi.org/10.1016/S0966-842X\(03\)00143-4](http://dx.doi.org/10.1016/S0966-842X(03)00143-4)
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 2012;28:1647–9. <http://dx.doi.org/10.1093/bioinformatics/bts199>
- Kosoy M, Bai Y, Lynch T, Kuzmin IV, Niezgoda M, Franka R, et al. *Bartonella* spp. in bats, Kenya. *Emerg Infect Dis*. 2010;16:1875–81. <http://dx.doi.org/10.3201/eid1612.100601>
- Bai Y, Kosoy M, Recuenco S, Alvarez D, Moran D, Turmelle A, et al. *Bartonella* spp. in bats, Guatemala. *Emerg Infect Dis*. 2011;17:1269–72. <http://dx.doi.org/10.3201/eid1707.101867>
- Brook CE, Bai Y, Dobson AP, Osikowicz LM, Ranaivoson HC, Zhu Q, et al. *Bartonella* spp. in fruit bats and blood-feeding ectoparasites in Madagascar. *PLoS Negl Trop Dis*. 2015; 9:e0003532. <http://dx.doi.org/10.1371/journal.pntd.0003532>
- Kosoy M, Morway C, Sheff KW, Bai Y, Colborn J, Chalcraft L, et al. *Bartonella tamiae* sp. nov., a newly recognized pathogen isolated from three human patients from Thailand. *J Clin Microbiol*. 2008;46:772–5. <http://dx.doi.org/10.1128/JCM.02120-07>
- Loan HK, Van Cuong N, Takhampunya R, Klangthong K, Osikowicz L, Kiet BT, et al. *Bartonella* species and trombiculid mites of rats from the Mekong Delta of Vietnam. *Vector Borne Zoonotic Dis*. 2015;15:40–7. <http://dx.doi.org/10.1089/vbz.2014.1604>

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Seropositivity for Avian Influenza H6 Virus among Humans, China

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To the Editor: Influenza virus subtype H6 was first isolated from a turkey in 1965 in the United States (1) and was subsequently found in other parts of the world (2). Over the past several decades, the prevalence of H6 virus has dramatically increased in wild and domestic birds (2–4). In China, highly pathogenic influenza A(H5N1), low pathogenicity influenza (H9N2), and H6 are the most prevalent avian influenza viruses among poultry (5). Although only 1 case of H6 virus infection in a human has been reported worldwide (6), several biological characteristics of H6 viruses indicate that they are highly infectious to mammals. Approximately 34% of H6 viruses circulating in China have enhanced affinity to human-like receptors (α -2,6 NeuAcGal) (2). H6 viruses can also infect mice without prior adaptation (2,7), and some H6 viruses can be transmitted efficiently among guinea pigs (2). To evaluate the potential threat of H6 viruses to human health, we conducted a systematic serologic study in populations occupationally exposed to H6 viruses.

During 2009–2011, a total of 15,689 serum samples were collected from live poultry market workers, backyard poultry farmers, large-scale poultry farmers, poultry-slaughter factory workers, and wild bird habitat workers in 22 provinces in mainland China. A/chicken/Y94/Guangdong/2011 (H6N2), a representative isolate of predominant H6 viruses in mainland China, was used for the serologic testing (online Technical Appendix Table 1, Figures 1, 2, <http://wwwnc.cdc.gov/EID/article/21/7/15-0135-Techapp1.pdf>). Hemagglutination inhibition (HI) assay was performed for all serum samples, and samples with an HI titer ≥ 20 were verified by a microneutralization (MN) assay, as indicated by World Health Organization guidelines (8). An MN result of ≥ 20 was considered positive.

The HI result was ≥ 20 for H6N2 virus in 298 of the 15,689 specimens, and the MN result was positive in 63 of the 298 specimens (overall seropositivity range 20–320, mean 32.7, 0.4%) (online Technical Appendix Table 2). The proportion of group members who were seropositive differed significantly according to occupational exposure ($p = 0.0125$). Seropositivity was highest among workers in live poultry markets, backyard poultry farmers, and workers in wild bird habitats (0.66%, 0.42%, and 0.51%, respectively) (Table). According to χ^2 test results, seropositivity among workers in live poultry markets was significantly higher than that among large-scale poultry farmers ($p = 0.0015$, adjusted $\alpha = 0.005$). Analysis by unconditional logistic regression model showed that exposure to live poultry markets was a risk factor for human infection with avian influenza H6 virus (odds ratio 2.1, 95% CI 1.27–3.47).

Seropositivity did not differ significantly among male and female persons tested ($p = 0.08$) (Table). No children were positive for the H6N2 virus. For other age groups, seropositivity ranged from 0.25% to 0.45%, but differences were not significant ($p > 0.05$) (Table).

Of the 22 provinces from which serum specimens were collected, 11 were northern provinces and 11 were southern provinces. Positive specimens were detected in all southern provinces. In northern China, no seropositive results were detected in Henan, Liaoning, or Jilin Provinces. According to χ^2 test results, seropositivity in southern China was significantly higher than seropositivity in northern China ($p = 0.0375$) (Table).

Human infection with influenza H6 virus in mainland China has not been reported, but 63 serum specimens tested in our study were positive for the H6 virus. This level of seropositivity is much higher than that for highly pathogenic

Table. Seropositivity of occupationally exposed populations for the influenza (H6N2) virus, China, 2009–2011*

Population	Total no. serum samples	Mean titer for MN ≥ 20	No. serum samples with MN ≥ 20	Seropositivity (95% CI)	Odds ratio† (95% CI)
Total	15,689	32.70	63	0.40 (0.40–0.41)	
Occupation					
Live poultry market	3,950	43.08	26	0.66 (0.64–0.68)	2.10 (1.27–3.47)
Poultry farm	3,762	25.71	7	0.19 (0.18–0.19)	0.40 (0.18–0.87)
Backyard poultry farm	4,324	26.67	18	0.42 (0.40–0.43)	1.05 (0.61–1.82)
Poultry slaughter factory	1,235	30.00	2	0.16 (0.15–0.17)	0.38 (0.09–1.57)
Wild bird habitat	788	20.00	4	0.51 (0.47–0.54)	1.28 (0.47–3.54)
Other	1,630	23.33	6	0.37 (0.35–0.39)	0.91 (0.39–2.11)
Sex					
F	7,620	24.29	28	0.37 (0.36–0.38)	Reference
M	8,069	39.39	35	0.43 (0.42–0.44)	1.18 (0.72–1.94)
Age group, y					
Children, ≤ 14	74	–	0	0	0 (0)
Youth, 15–24	1,168	20.00	3	0.26 (0.24–0.27)	0.75 (0.19–3.00)
Adult, 25–59	1,2450	34.07	54	0.43 (0.43–0.44)	1.27 (0.54–2.94)
Elderly, ≥ 60	1,748	13.33	6	0.34 (0.33–0.36)	Reference
No age record	249	–	0	0	–
Geographic distribution					
South	10,522	32.00	50	0.48 (0.47–0.48)	Reference
North	5,167	35.38	13	0.25 (0.24–0.26)	0.59 (0.30–1.15)

*MN, microneutralization; –, not applicable.

†Odds ratios were calculated by using unconditional logistic regression model (SPSS 17.0, Armonk, NY, USA).

avian influenza A(H5N1) virus, for which only 2 of the serum specimens we tested were positive (data not shown), but much lower than the seropositivity level for low pathogenicity avian influenza A(H9N2) virus; 3.4% of the samples tested were positive for A/Chicken/Hong Kong/G9/1997(H9N2)-like virus (data not shown). A previous US study has reported H6N2-positive antibodies in veterinarians (9). Our results and the veterinarian study indicate that the H6N2 virus could infect humans.

In our study, positive samples were detected in 19 of 22 provinces and in all tested worker populations, suggesting that the H6 virus has been broadly circulating in birds in China. Live poultry market exposure is the major risk factor for human infection with avian influenza H6 virus. The limitation of this study is that antigen selection may not accurately detect neutralization antibodies for different subtypes of H6 viruses. Surveillance of the H6 virus in birds and occupationally exposed populations should be strengthened for pandemic preparedness.

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References

- Downie JC, Webster RG, Schild GC, Dowdle WR, Laver WG. Characterization and ecology of a type A influenza virus isolated from a shearwater. *Bull World Health Organ.* 1973;49(6):559-66.
- Wang G, Deng G, Shi J, Luo W, Zhang G, Zhang Q, et al. H6 influenza viruses pose a potential threat to human health. *J Virol.* 2014;88:3953–64. <http://dx.doi.org/10.1128/JVI.03292-13>
- Jiao P, Yuan R, Wei L, Jia B, Cao L, Song Y, et al. Complete genomic sequence of a novel natural recombinant H6N2 influenza virus from chickens in Guangdong, Southern China. *J Virol.* 2012;86:7717–8. <http://dx.doi.org/10.1128/JVI.00963-12>
- Zhao G, Lu X, Gu X, Zhao K, Song Q, Pan J, et al. Molecular evolution of the H6 subtype influenza A viruses from poultry in eastern China from 2002 to 2010. *Virol J.* 2011;8:470. <http://dx.doi.org/10.1186/1743-422X-8-470>
- Pepin KM, Wang J, Webb CT, Smith GJ, Poss M, Hudson PJ, et al. Multiannual patterns of influenza A transmission in Chinese live bird market systems. *Influenza Other Respir Viruses.* 2013;7:97–107. <http://dx.doi.org/10.1111/j.1750-2659.2012.00354.x>
- Yuan J, Zhang L, Kan X, Jiang L, Yang J, Guo Z, et al. Origin and molecular characteristics of a novel 2013 avian influenza A(H6N1) virus causing human infection in Taiwan. *Clin Infect Dis.* 2013;57:1367–8. <http://dx.doi.org/10.1093/cid/cit479>
- Gillim-Ross L, Santos C, Chen Z, Aspelund A, Yang CF, Ye D, et al. Avian influenza H6 viruses productively infect and cause illness in mice and ferrets. *J Virol.* 2008;82:10854–63. <http://dx.doi.org/10.1128/JVI.01206-08>
- World Health Organization. Manual for the laboratory diagnosis and virological surveillance of influenza. Geneva: The Organization; 2011. p. 63–77.
- Myers KP, Setterquist SF, Capuano AW, Gray GC. Infection due to 3 avian influenza subtypes in United States veterinarians. *Clin Infect Dis.* 2007;45:4–9. <http://dx.doi.org/10.1086/518579>

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Absence of MERS-Coronavirus in Bactrian Camels, Southern Mongolia, November 2014

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To the Editor: Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified among humans in 2012 in Saudi Arabia (1). As of February 5, 2015, a total of 971 MERS cases and 356 associated deaths had been confirmed (2). Because MERS is a zoonotic disease, it is essential that the animal reservoirs and hosts that sustain virus circulation in nature be identified.

Seroepidemiologic and virologic studies have demonstrated evidence of MERS-CoV infection in dromedary camels (*Camelus dromedarius*) in the Arabian Peninsula (3), and viruses isolated from dromedaries appear capable of infecting the human respiratory tract (4). In some instances, MERS-CoV infection in dromedaries has preceded infection in humans (5), indicating that dromedaries are a natural host for MERS-CoV and a possible source of human infection. Thus, it is important to define the geographic range of MERS-CoV infection in camels and the species of camelids that are infected by MERS-CoV in nature.

Two species of camels exist: 1-hump dromedaries (*C. dromedarius*) and 2-hump Bactrian camels (*C. bactrianus*).

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Seropositivity for Avian Influenza H6 Virus among Humans, China

Technical Appendix

Technical Appendix Table 1. Levels of neutralization antibodies from serum specimens of occupationally exposed populations for the avian influenza (H6N2) virus, China*

Characteristic	Serum specimens	Serum specimens, MN \geq 20 no. (%)	Serum specimens, MN \geq 40 no. (%)	Serum specimens, MN \geq 80 no. (%)	Serum specimens, MN \geq 160 no. (%)	Serum specimens, MN = 320 no. (%)
Total population	15,689	63 (0.40)	14 (0.09)	5 (0.03)	2 (0.01)	1 (0.01)
Occupation						
Live poultry market worker	3,950	26 (0.66)	6 (0.15)	4 (0.10)	2 (0.05)	1 (0.03)
Poultry farmer	3,762	7 (0.19)	2 (0.05)	0 (0)	0 (0)	0 (0)
Backyard poultry farmer	4,324	18 (0.42)	4 (0.09)	1 (0.02)	0 (0)	0 (0)
Poultry-slaughter factory worker	1,235	2 (0.16)	1 (0.08)	0 (0)	0 (0)	0 (0)
Wild bird habitat worker	788	4 (0.51)	0 (0)	0 (0)	0 (0)	0 (0)
Others	1,630	6 (0.37)	1 (0.06)	0 (0)	0 (0)	0 (0)
Gender						
Female	7,620	28 (0.37)	4 (0.05)	1 (0.01)	0 (0)	0 (0)
Male	8,069	35 (0.43)	10 (0.12)	4 (0.05)	2 (0.02)	1 (0.01)
Age groups						
Children (\leq 14)	74	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Youth (15–24)	1,168	3 (0.26)	0 (0)	0 (0)	0 (0)	0 (0)
Adult (25–59)	12,450	54 (0.43)	12 (0.10)	5 (0.04)	2 (0.02)	1 (0.01)
Elderly (\geq 60)	1,748	6 (0.34)	2 (0.11)	0 (0)	0 (0)	0 (0)

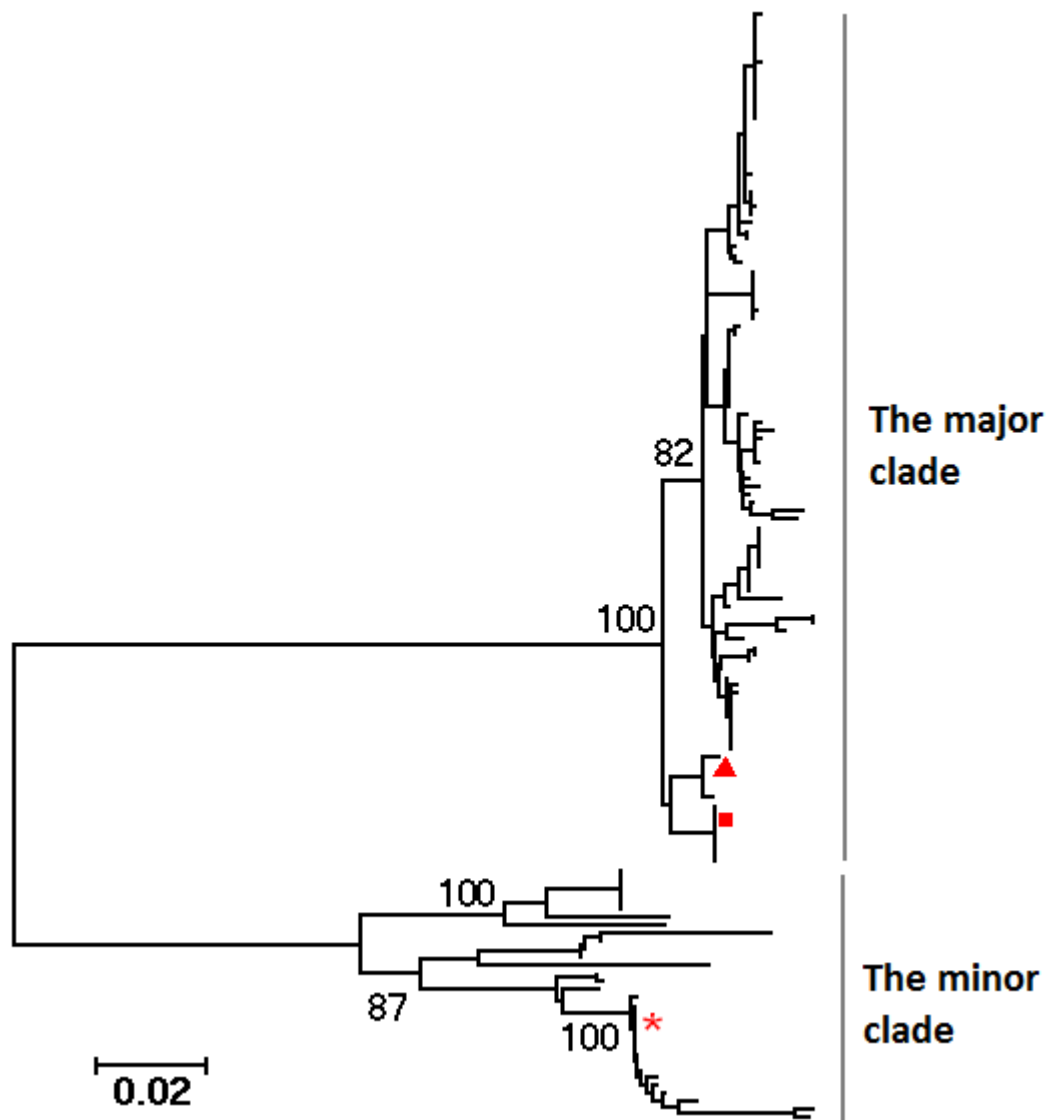
Characteristic	Serum specimens	Serum specimens,	Serum specimens,	Serum specimens,	Serum specimens,	Serum specimens,
		MN ≥20 no. (%)	MN ≥40 no. (%)	MN ≥80 no. (%)	MN ≥160 no. (%)	MN = 320 no. (%)
No age record	249	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Geographic distribution						
South	10,522	50 (0.48)	10 (0.10)	4 (0.04)	1 (0.01)	1 (0.01)
North	5,167	13 (0.25)	4 (0.08)	1 (0.02)	1 (0.02)	0 (0)

*MN, microneutralization; specimens were tested by using MN assay.

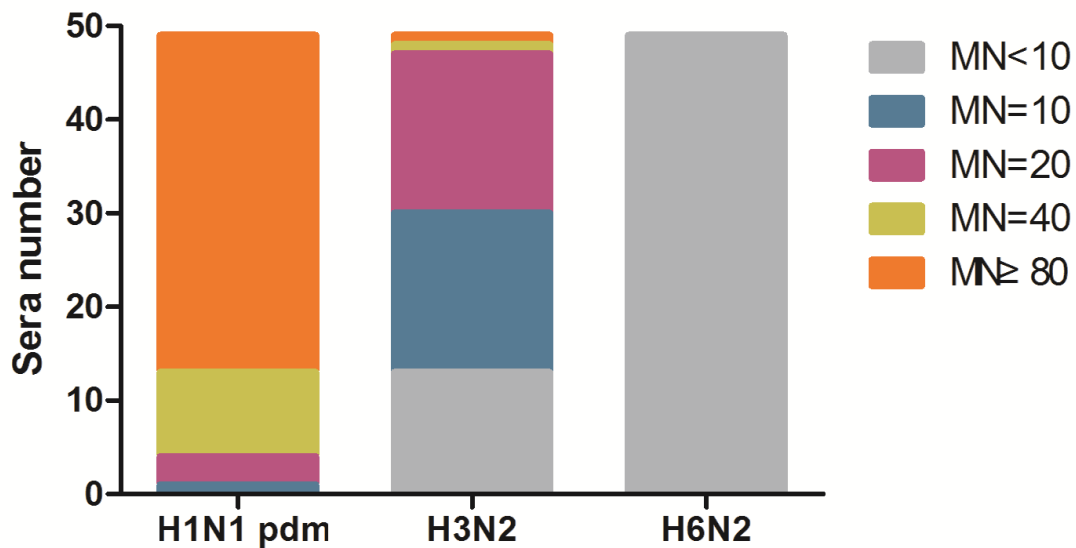
Technical Appendix Table 2. Antigenic analysis of randomly selected H6N2 viruses and an H9N2 virus circulating in China*

Virus Strain	Ferret antisera		
	A/chicken/Y94/Guangdong/2011	A/chicken/YF6/Guangdong/2011	A/chicken/AK4/Anhui/2011
A/chicken/Y94/Guangdong/2011(H6N2)	2,560	2,560	<10
A/chicken/ YF6/Guangdong/2011(H6N2)	2560	5,120	<10
A/chicken/AK4/Anhui/2011(H9N2)	<10	<10	10,240

*Antigenic analysis was performed with hemagglutination assay by using 1% turkey red blood cells. Two representative avian influenza (H6N2) viruses located in the major clade and 1 avian influenza (H9N2) virus were randomly selected. Ferret antisera raised against these 3 viruses were used. No cross reaction occurred between H9N2 and H6N2 viruses. The 2 H6N2 viruses were antigenically similar. Homologous titers are in bold.



Technical Appendix Figure 1. Phylogenetic tree of H6 avian influenza viruses circulating in poultry in China in 2011 on the basis of HA1 domain sequences. Of 142 H6 subtype viruses isolated from birds in China in 2011, 140 were sequenced and classified into 2 clades. ▲ represents *A/chicken/Guangdong/Y94/2011(H6N2)*, ■ represents *A/chicken/Guangdong/YF6/2011(H6N2)*, and * represents *A/environment/Hunan-changsha/14/2011*. Marked viruses were randomly selected for antigenic analysis. The phylogenetic tree was generated by the neighbor-joining method using Mega 6.0 (<http://www.megasoftware.net>). The bootstrap values of the main branch are shown. The scale bar indicates nucleotide substitutions per site.



Technical Appendix Figure 2. Cross reaction of seasonal H1N1 pdm and H3N2 viruses with the H6N2 avian influenza virus by MN assay. Of sera samples positive for seasonal influenza H1N1 pdm and H3N2 viruses by HI assay, 49 were randomly selected for a cross-reaction analysis by using the MN assay. Results showed that sera testing positive for H1N1 pdm or H3N2 all had an MN titer <10 for H6N2, indicating no cross-reactions between H6N2 avian influenza and H1N1 pdm/H3N2 viruses. The H1N1pdm, H3N2, and H6N2 antigens used were A/California/07/2009(H1N1), A/Brisbane/10/2007(H3N2), and A/chicken/Y94/Guangdong/2011(H6N2), respectively. MN, microneutralization.