Short Communication

First Positive Detection of H9 Subtype of Avian Influenza Virus Nucleic Acid in Aerosol Samples from Live Poultry Markets in Guangxi, South of China

Ning Kang, Min Chen, Fu-Yin Bi, Min-Mei Chen, Yi Tan

Institute of Acute Infectious Diseases Control and Prevention, Guangxi Zhuang Autonomous Region for Disease Prevention and Control, Nanning, Guangxi 530028, China

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Introduction

Avian influenza has become a serious public health problem. Risk factors for human cases are direct or closed contact with ill, died poultry and live poultry markets (LPMs) exposure. There is high risk of infection with high frequency of visiting LPMs.[1] Robust data of human and animal models have provided support for the potential for influenza virus transmission by aerosol. The experimental data showed that aerosol transmission accounts for approximately half of influenza A virus spread events.[2] Thus, identifying the circulating influenza viruses in environmental samples, especially in aerosol of LPMs, is necessary for evaluating public health threat and taking preventive measure. The present study is aimed to analyze the distribution of avian influenza viruses (AIVs) in aerosol and other styles of environmental samples.

Methods

There are hundreds of LPMs in Nanning, which opened daily. We collected samples in the Langxi LPM, which were one of the biggest and famous LPMs with 12 stalls in Qingxiu district. Aerosol samples were collected in one random stall (including chickens, geese, and ducks) among 12 stalls every week. They were collected by SKC SETI BioSampler (SKC, US PATENT #5,902,385)[3] at 1.5 m high in 9 ml viral sampling medium (Yocon MT0301-1, China) for 1h with 12 L/min. At the same time, floor sewage, swab samples of poultry cage and chopping block, and feather were collected and stored at 4°C. Viral RNA was extracted from the specimens using the viral RNA mini kit (Qiagen 74104, Germany). Real-time polymerase chain reaction was performed with influenza A virus nuclei acid detected kit (BioPerfectus Technologies, JC10103, China). Samples tested positive for influenza A viral RNA were then analyzed by influenza H5/H7/H9 subtype nuclei acid detected kit (BioPerfectus Technologies, JC10301, China). Serum specimens of occupational population were collected and detected antibody levels for H5N1 and H7N9 by hemagglutination inhibition tests, respectively.

Data were entered into a customized database (Microsoft Excel 2007, Microsoft, USA) and then transferred into SPSS (version 16.0,SPSS Inc., Chicago, IL, USA) for analysis.

Results

Overall, 699 of 1526 (45.81%) samples were positive for influenza A nuclei acid [Table 1]. Influenza A nuclei acid was detected positive in all kinds of the environmental samples. We found that the positive rate was significant difference between distinct style samples ($\chi^2 = 51.373$, $P = 0.000$ for 2013; $\chi^2 = 32.219$, $P = 0.000$, for 2014).

The research found that 30 of 75 (40.00%) aerosol samples were positive for influenza A nuclei acid, in which six samples (20.00%) were positive for H9 subtypes of AIV

Address for correspondence: Dr. Yi Tan,
Institute of Acute Infectious Diseases Control and Prevention, Guangxi Zhuang Autonomous Region Center for Disease Prevention and Control, Nanning, Guangxi 530028, China
E-Mail: tanyigxcdc@126.com

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Table 1: Results of real-time RT-PCR testing for influenza A in environmental samples collected from LPMs, Nanning, China, April 2013 to December 2014

Year of collection	Sample styles	RT-PCR testing		Influenza virus A subtyping, *n* (%)			
		Number of tested, *n*	Number of positive, *n* (%)	H5	H7	H9	Other
2013	Aerosol	38	14 (36.84)	0 (0)	0 (0)	0 (0)	14 (100)
	Sewage	198	104 (52.53)	31 (29.81)	0 (0)	13 (12.50)	60 (57.69)
	Poultry cage	198	67 (33.84)	4 (5.97)	0 (0)	8 (11.94)	55 (82.09)
	Chopping block	198	51 (25.76)	17 (33.33)	0 (0)	4 (7.84)	30 (58.82)
	Feather	122	43 (35.25)	15 (34.88)	0 (0)	2 (4.65)	26 (60.47)
	Total	754	279 (37.00)	67 (24.01)	0 (0)	27 (9.68)	185 (66.31)
2014	Aerosol	37	16 (43.24)	0 (0)	0 (0)	6 (37.50)	10 (62.50)
	Sewage	184	137 (74.46)	32 (23.60)	0 (0)	65 (47.45)	40 (29.20)
	Poultry cage	184	71 (38.59)	7 (9.86)	0 (0)	39 (54.93)	25 (35.21)
	Chopping block	183	93 (50.82)	18 (19.35)	0 (0)	36 (38.71)	39 (41.94)
	Feather	184	103 (55.98)	22 (21.36)	0 (0)	40 (38.83)	41 (39.81)
	Total	772	420 (54.40)	79 (18.81)	0 (0)	186 (44.29)	155 (36.90)
2013–2014	Summary	1526	699 (45.81)	146 (20.89)	0 (0)	213 (30.47)	340 (48.64)

RT-PCR: Reverse transcription-polymerase chain reaction; LPMs: Live poultry markets.

nuclei acid and 24 (80.00%) were positive for other subtypes [Table 1]. Except for aerosol samples, other environmental samples were positive for both H5 and H9 subtypes [Table 1]. There was a higher positive rate for H5 subtypes than H9 subtypes among positive influenza A virus samples in 2013 ($\chi^2 = 20.47$, $P < 0.01$), in contrast, a higher positive rate for H9 subtypes than H5 subtypes among positive influenza A virus samples in 2014 ($\chi^2 = 63.12$, $P < 0.01$).

No sample was positive for H7 subtype of AIV. As shown in Figure 1a, the distribution of influenza A H5, H9 subtype and other influenza A subtypes had a great diversity in each month.

There was significant difference of positive rate for influenza A virus monthly for both 2013 and 2014 (for 2013, $\chi^2 = 84.272$, $P = 0.000$; for 2014, $\chi^2 = 72.751$, $P = 0.000$) with a higher positive rate in the autumn and winter [Figure 1b], which was consistent with previous results.

It was found that 840 and 470 serums specimens were negative for H5N1 and H7N9 antibody, which were collected from occupational population in 2013 and 2014, respectively.

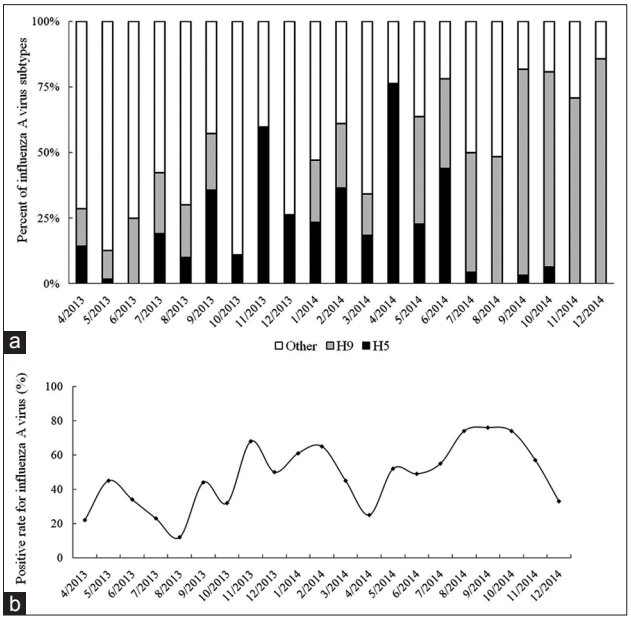

Figure 1: (a) Percent of Influenza virus A H5/H7/H9 subtypes monthly during April 2013 to December 2014. (b) Positive rate for influenza A of environmental samples monthly from April 2013 to December 2014.

Discussion

Overall, the present results suggest that AIV nucleic acid exist in aerosol and other environmental samples from LPMs of Guangxi. Aerosol transmission might be an important mode of human infected by AIV after visiting LPMs; thus, it is needed to monitor virus distribution in aerosol sample from LPMs. Previous studies have shown that LPMs were closely linked to H9N2, H5N1, and H7N9 infection in human in 2003,[4] 2006, and 2013,[5] respectively.

LPMs promote the transmission of AIV from avian to human. It is still needed to confirm the transmitted mechanism through exposure or visiting LPMs. Chickens were successfully infected with AIV H5N1and H9N2 by aerosol and caused higher titers of virus.[6,7] Avian influenza H9 has been isolated animals from LPMs[8-11] and from air in chicken houses in China.[12] Our results first reported H9 subtype of AIV nucleic acid existing in aerosol sample from LPM of Guangxi, China, providing support for aerosol transmission of AIV in LPM.

H5 subtype of AIV nucleic acid was not detected positive in aerosol while it was positive in other four styles of environmental samples. H9 subtype was positive in aerosol and other four styles of environmental samples in 2014. It is still needed further study to investigate whether there are differences in viral viability between H5 and H9 subtypes in aerosol and other environmental samples.

As LPMs play an important role in the dissemination of AIVs, active surveillance to monitor AIV in LPMs should be carried out as an early warning system for AIV outbreaks.

The widespread distribution of H9 in poultry and its potential for reassortment are a credible threat for human infection in the future.[9]

No AIV had been isolated from AIV nucleic acid positive aerosol specimens. The possible reasons are low survival and concentration of AIV in aerosol collected by BioSampler.

The results of serological surveillance for 2 years suggested that no recessive or previous infection of AIVs was found in occupational population, although AIVs prevailed in the environment of PLM in Guangxi, which was consistent with previous results of Guangxi in recent years. It implies that the potential for AIV infect human is limited.

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Conflicts of interest

There are no conflicts of interest.

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