Avian Influenza Outbreaks and Surveillance in Live Bird Markets, Quang Ninh Province, Vietnam, 2015-2017

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Abstract

Over 5,000 outbreaks of avian influenza (AI) have occurred in Vietnam, with more than 60 million birds infected and destroyed from 2003 to 2015. This study aimed to describe the AI situation and associated risk factors after 2015. Outbreaks, surveillance and molecular characteristics data in Quang Ninh Province from 2015 to 2017 were gathered from Regional Animal Health Office Number 2. Risk factors for AI virus found in live bird markets (LBMs) were identified using odds ratios (OR) with 95% level of confidence. Ten outbreaks of AI were reported in the border area between Quang Ninh Province and China. The AI active surveillance detected viruses in LBMs from 37.3% (227/608) of the samples. Of these, 7.0% (16/227) were H5N6, and all 608 samples were negative for H5N1 and H7 subtypes. Poultry at LBMs in Quang Ninh imported from Bac Giang Province was slightly more likely to be infected with AI (OR = 1.4, 95% CI = 1.01-2.04). Provincial and national animal health authorities should continue to conduct active surveillance and strictly enforce poultry movement control from China as well as stop transportation of infected poultry across provinces in Viet Nam.

Keywords: avian influenza, H5N6, live bird market, Quang Ninh, Vietnam

Introduction

Avian influenza (AI) is a zoonotic disease, and can cause severe illness and death in humans and poultry. Many subtypes of AI are spreading globally. The identified subtypes of influenza H5 include H5N1, H5N2, H5N6, H5N8 and H5N9.[1] Since the beginning of influenza A(H5N1) epidemic in 2003 till March 2017, hundreds of millions of birds have died[1] and 453 human fatalities in 16 countries were attributable to H5N1 virus[2]. Influenza A(H7N9) was firstly reported in Shanghai, China, during 2013, and associated human cases were found in other territories such as Hong Kong, Taiwan, Canada and Malaysia.[3] From March 2013 to February 2018, a total of 1,624 people were infected with influenza A(H7N9) virus in China, resulting in 621 deaths.[3]

Highly pathogenic avian influenza (HPAI) subtype H5N1 first appeared in Vietnam during late December 2003. By 2015, there were over 5,000 outbreaks in poultry, with more than 60 million birds infected and

destroyed. Consequentially, 127 people were infected with influenza A(H5N1), including 64 deaths.[4] Vietnamese consumers prefer to buy live poultry from live bird markets (LBMs) and slaughter them for consumption. Several poultry species are sold at LBMs, including ducks, chickens, geese and quails. LBM is a location where AI viruses can accumulate, multiply and spread the infection. In addition, the majority of LBMs are lack of appropriate waste management system. Poultry vendors usually work without personal protection such as masks and gloves. The remaining poultry are kept overnight and mixed with new batch of poultry arriving each day.

Quang Ninh Province is a tourist attraction that shares a border with China, and high demand for live poultry exists in the province while the supply is limited. High capacity of poultry imported from other provinces and China poses a risk of AI outbreaks in the province. Both national and provincial animal health authorities required better understanding on AI situation for better control and prevention. Hence, this study aimed to describe the occurrence of AI from outbreak reports and LBM surveillance, and assess risk factors for AI in Quang Ninh Province from 2015 to 2017.

Methods

Information from the AI surveillance at LBMs and the outbreak reports from the Regional Animal Health Office 2 (RAHO2) were compiled into a single database for analysis, including details of outbreaks reported by provincial animal health office in Quang Ninh, and laboratory and sequencing results tested by the laboratory section of RAHO2.

In Quang Ninh Province, there were 26 registered LBMs that each had a volume of at least 100 birds sold per day. Of 26 LBMs, 10 were randomly selected and samples were collected once a month during the study period (Figure 1). In each market, the sample collectors selected six vendors. Five poultry swabs from chickens and ducks, and other environmental samples such as fresh feces, waste in the cage, waste water outside the cage and drinking water from each vendor were collected. The samples were obtained for four rounds (A to D) during 2015-2017 (Table 1). At the time of sample collection, the sample collectors used a standard questionnaire to gather information from the vendor on risk factors as well.
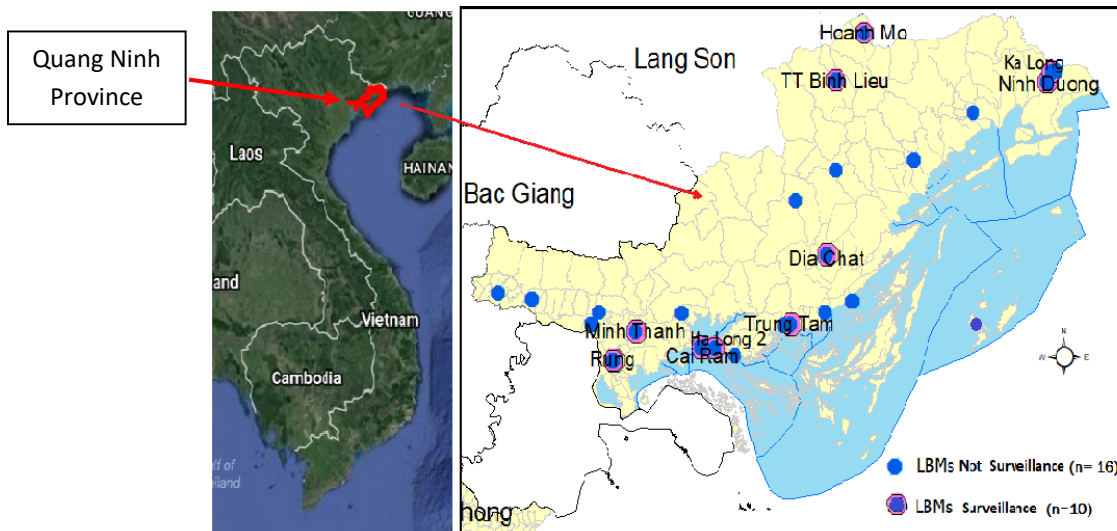

Figure 1. Location of over 100 birds sold and randomly selected live bird markets for avian influenza surveillance in Quang Ninh Province, Vietnam, 2015-2017

Table 1. Samples collected under the avian influenza surveillance at live bird markets (LBMs) in Quang Ninh Province, Vietnam, 2015-2017 (n=608)

| Round | Period | LBM | Month | Pooled samples by type (per LBM/time) | | | Total pool sample |
| | | | | Throat swab | | Environmental swab | |
				Chicken	Duck		
A	Dec 2015 - Feb 2016	4	3	6	6	6	216
B	Jun - Aug 2016	4	3	6	6	6	216
C	Mar - May 2017	2	3	-	-	8	128
		2	2	-	-	8	
		2	2	12	-	-	
D	Jun - Aug 2017	2	3	-	-	8	48

Five cotton swabs from the similar type of sample or similar species were pooled into one tube and tested for AI. If the sample was positive to influenza A, it was then tested for H5, N1, N6, H7 and H9 by reverse transcriptase polymerase chain reaction (RT-PCR). The tests were performed in the laboratory of RAHO2 using a standard protocol for AI surveillance[5]. Furthermore, two H5N6 viruses from outbreaks were selected for molecular sequencing and the HA gene was amplified by PCR[6]. The amplified products were later sent to the Macrogens Company in the Republic of Korea for sequencing. Phylogenetic analysis on HA sequences, including sequences downloaded from Genbank, was conducted using MEGA 6.0 with neighbor-joining, bootstrap 1000 replications[7].

The results were described using descriptive statistics. ArcGIS 9.3 program[8] was used to create a distribution map. Epicals 2000 program[9] was also used to calculate odds ratios (OR) with 95% confidence level (CI) to determine the association between AI and potential risk factors, including poultry source (province), poultry species and types of sample.

Results

HPAI Outbreaks

From 2015 to 2017, 10 HPAI outbreaks were reported in five out of 14 districts of Quang Ninh Province. These outbreaks could be grouped into three waves: wave 1 from October to December 2015 caused by H5N6 virus, wave 2 from October 2016 to January 2017 caused by H5N6 virus, and wave 3 in April 2017 caused by H5N1 virus (Figure 2). All outbreaks occurred in the areas with moderate to high density of poultry population (Figure 3).
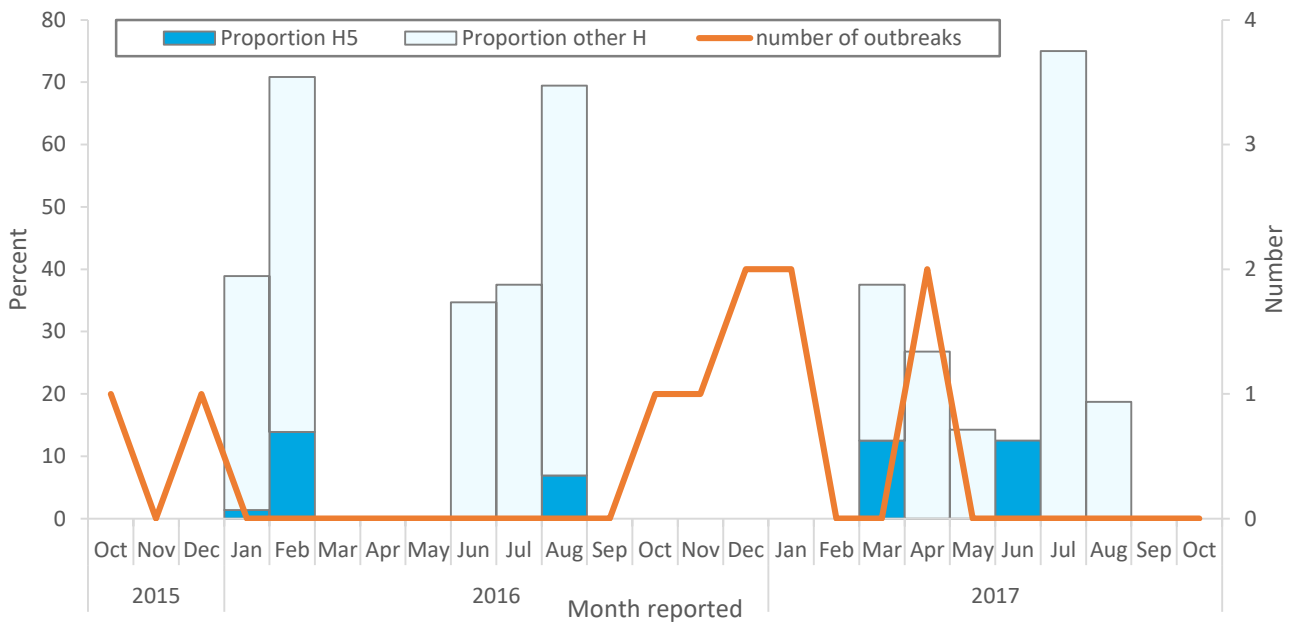

Figure 2. Avian influenza subtypes detected by surveillance and causing outbreaks in Quang Ninh Province, Vietnam, October 2015 to August 2017
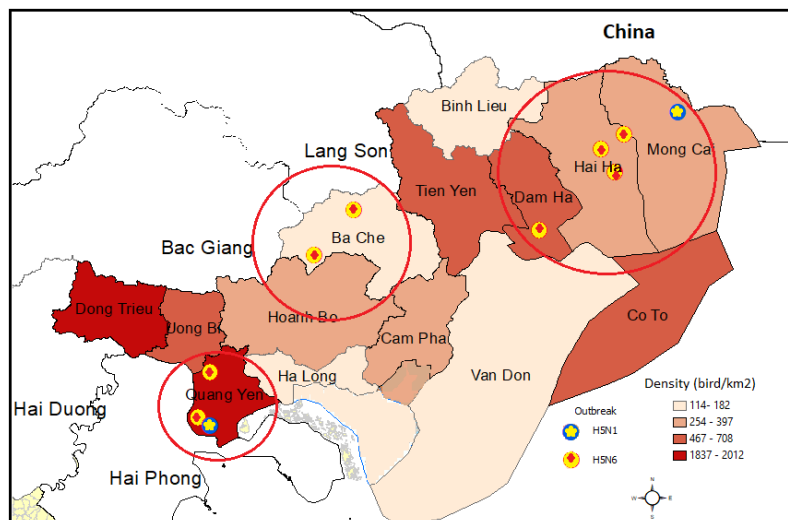

Figure 3. Location of highly pathogenic avian influenza outbreaks in Quang Ninh Province, Vietnam, October 2015 - August 2017 (n=10)

AI Surveillance at LBMs

A total of 608 pool samples were collected from LBMs from 2015 to 2017. Of which, the majority (66.0%) of poultry swab samples were collected from poultry produced in Quang Ninh Province while others were collected from poultry produced in Bac Giang (31.7%), Nam Dinh (1.3%) and Hai Duong (1.0%) Provinces. Throat swabs made up to 55.3% (336/608) of the samples while 44.7% (272/608) of all samples were collected from the environment. The proportion of AI virus was found to be 37.3% (227/608) in LBMs during 2015-2017 (Table 2).

A high proportion of samples were positive for AI in round B (89.5%, 102/114), followed by round A (57.7%, 79/137), and round D (54.8%, 17/31), with the lowest in round C (29.3%, 29/99). About 34.9% (95/272) of environmental samples and 39.3% (132/336) of throat swabs samples were found to have AI infection. Further characterization showed that 7.0% (16/227) of the samples positive for influenza A were H5N6. Nine

out of AI positive samples collected during April-May 2017 were selected and four (44.4%) out of nine samples were found to have H9. All 608 samples were negative for H5N1 and H7 (Table 2). HPAI virus occurrence in LBM was significantly associated with poultry coming from Bac Giang Province (OR = 1.4, 95% CI = 1.01-2.04) (Table 3).

The surveillance system in LBMs detected AI in every month during the sampling period. HPAI H5N1 outbreaks were reported during the period when no AI was detected in the surveillance system (Figure 2).

Molecular Analysis

Two AI subtypes of H5N6 collected from outbreaks belonged to clade 2.3.4.4B. The virus circulating in Quang Ninh Province were closely related to the virus found in neighboring Lang Son Province. They were also closely related to AI viruses found in Dong Quan (H5N6), Sichuan (H5N1) and Shantou (H5N6) isolated in China during 2013 and 2014 (Figure 4).

Table 2. Laboratory results of avian influenza surveillance at live bird markets in Quang Ninh Province, Vietnam, 2015-2017 (n=608)

Type of sample		Number tested	Type A (%)	H5N6 (%)	H5N1 (%)	H7 (%)	H9 (%) (n=9)
Environmental swab	Drinking water	24	10 (41.7)	0	0	0	-
	Fresh feces	24	9 (37.5)	1 (11.1)	0	0	-
	Cage	176	59 (33.5)	4 (6.8)	0	0	-
	Waste water	48	17 (35.4)	1 (5.9)	0	0	-
	Total	272	95 (34.9)	6 (6.3)	0	0	-
Throat swab	Chicken	192	72 (37.5)	2 (2.8)	0	0	4 (44.4)
	Duck	144	60 (41.7)	8 (13.3)	0	0	-
	Total	336	132 (39.3)	10 (7.6)	0	0	-
Total		608	227 (37.3)	16 (7.0)	0	0	-

Table 3. Association between poultry source, species, type of sample and highly pathogenic avian influenza virus in Quang Ninh Province, Vietnam, 2015-2017

Variable	Total tested	Number positive	Percent	Odds ratio	95% CI	P-value
Poultry source (province)						
Bac Giang	193	84	43.5	1.4	1.01-2.04	0.05
Quang Ninh	401	140	34.9		Ref	
Poultry species						
Duck	144	60	41.7	0.9	0.60-1.35	0.68
Chicken	192	72	37.5		Ref	
Type of sample						
Throat swab	336	132	39.3	1.21	0.87-1.68	0.31
Environmental swab	272	95	34.9		Ref	
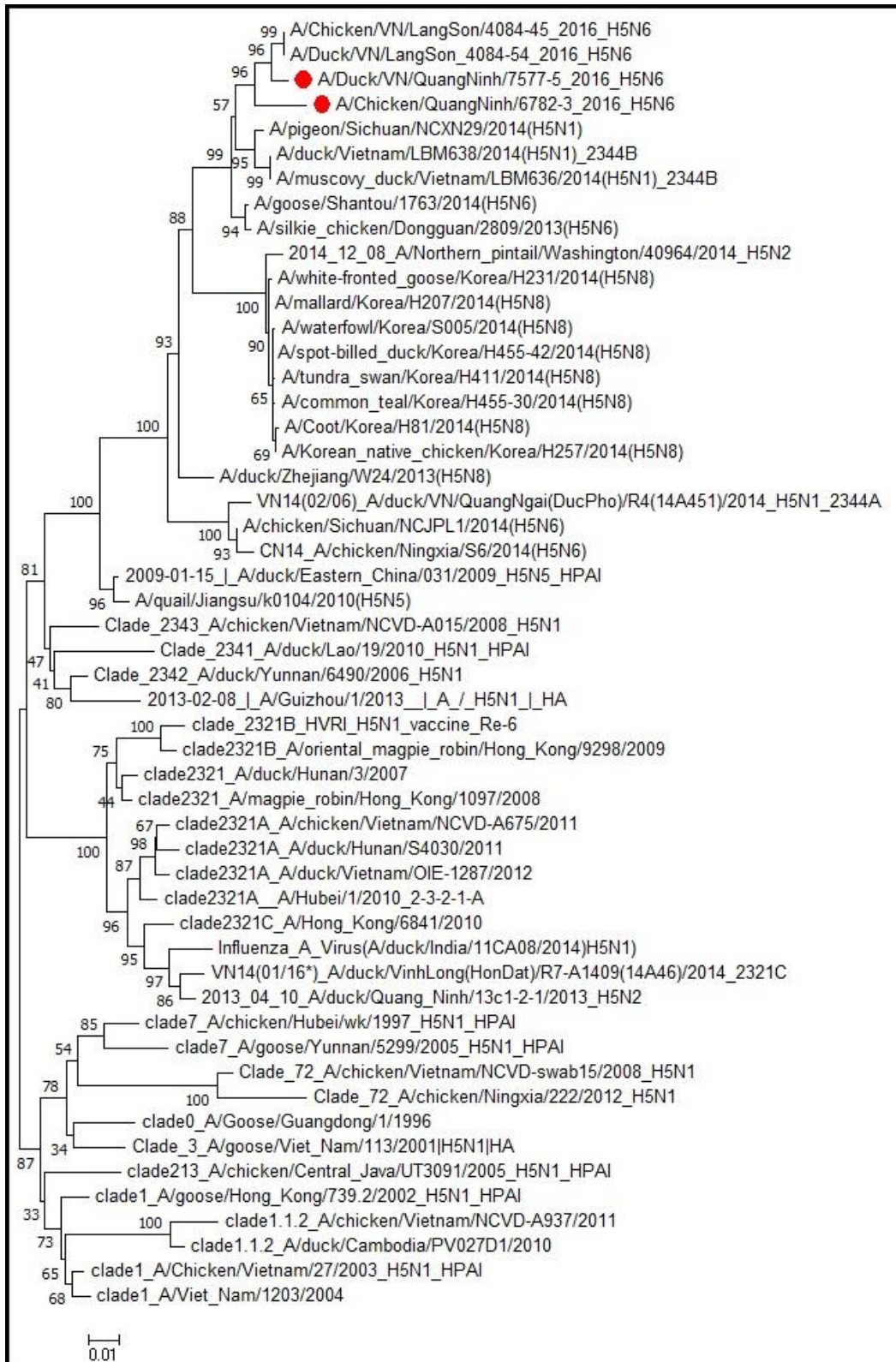
Figure 4. Phylogenetic analysis of HA genes from highly pathogenic avian influenza H5N6 detected in Quang Ninh Province, Vietnam, 2016

Discussion

Ten AI outbreaks among poultry were reported in Quang Ninh Province from 2015 to 2017, including eight outbreaks caused by H5N6 virus subtype and two by H5N1.

AI outbreaks reported during 2015-2017 occurred in the districts close to China, and Bac Giang Province which is connected to the western part of Quang Ninh Province and had higher poultry density (4,242 birds/km^2) than average in Vietnam (1,096 birds/km^2)[10]. A large amount of live poultry from China (80,000 tons per year) were imported to Vietnam for consumption due to the competitive price of live poultry.[11] Quang Ninh Province is the main entry of live poultry. Our molecular analysis showed that HPAI H5N6 virus was

shared in this region, regardless of country and provincial boundaries.

The proportion of AI virus found in LBMs from 2015 to 2017 (37.3%) was higher than the national average from 2011 to 2013 (22.1%, 2,162/9,790)[12]. Poultry sold at LBMs in Quang Ninh were imported from various areas, including nearby provinces and China, where poultry density is high. Poultry from Bac Giang Province were 1.4 times more likely to be infected with AI than poultry produced in Quang Ninh.

In LBMs, there were H5N6 subtype among influenza A positive samples and H9 in some selected samples while H7 and H5N1 subtypes were not detected. It had been shown earlier that H9 virus was a common donor of internal gene for other HPAI viruses.[13-16] Therefore, LBM could act as a mixing site for recombination among various influenza viruses which might result into a new HPAI subtype.

The fact that there was no HPAI H5N6 outbreaks during the study periods might reflect the under-reporting of HPAI outbreak from the local parties. AI surveillance in LBMs indicated that the AI viruses were circulated in the markets of Quang Ninh Province, and revealed a higher chance for genetic mutation and reassortment, including the risk of virus transmission to humans.

In conclusion, Quang Ninh Province was at risk for HPAI and HPAI viruses circulating in LBMs. The results of this study would be useful to improve short and long-term strategies for targeted surveillance at LBMs, relating to cross-border trade with neighboring provinces and China.

Limitations

The AI surveillance was not conducted at all LBMs in the province and the proportion might not represent the situation of AI in the whole province. In addition, the risk associated with the source of poultry might be due to sampling bias where the number of samples (n=193) collected from Bac Giang Province was much greater than Nam Dinh (n=8) and Hai Duong (n=6) Provinces.

The selected LBMs were those with more than 100 birds sold per day, in the area with crowded population, or located near the main road. Therefore, the proportion of positive AI results that we found in this study might be higher than the prevalence of all LBMs in the province.

Surveillance design aimed to detect AI in each selected LBM and was not designed specifically to determine risk factors for AI. Thus, this study was simply able to measure general risk factors rather than the specific

ones. However, the results could provide basis information to improve surveillance design, and control and prevention measures in the future.

Public Health and Policy Recommendations

As the HPAI H5N6 virus detected in LBMs was a potential risk for human infection, provincial health and market management authorities should enhance cleaning and disinfection at LBMs and encourage sellers to use face masks to protect themselves. While provincial public and animal health authorities should continue to conduct active surveillance and strictly enforce poultry movement control from China as well as prohibit the transportation of infected poultry across provinces in Vietnam.

Acknowledgements

Suggested Citation

Tran DT, Hoang BT, Pham VT, Chanachai K, Prarakamawongsai T, Padungtod P, et al. Avian influenza outbreaks and surveillance in live bird markets, Quang Ninh Province, Vietnam, 2015-2017. OSIR. 2018 Sep;11(3):1-7.

References

1. World Organisation for Animal Health. Update on avian influenza in animals (types H5 and H7): 2016. 2017 Sep 18. [cited 2018 Jan 24]. <http://www.oie.int/en/animal-health-in-the-world/update-on-avian-influenza/2016/>.

2. World Health Organization. Influenza at the human-animal interface: summary and assessment, 14 Feb to 16 Mar 2017 [cited 2018 Jan 24]. <http://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_03_16_2017.pdf>.

3. Food and Agriculture Organization of the United Nations. H7N9 situation update. 2018 February 14 [cited 2018 Jan 24]. <http://www.fao.org/ag/againfo/programmes/en/empres/h7n9/situation_update.html>.

4. Duong UT. What is H5N1 virus. 2017 Jul 18. Vietnamese [cited 2018 Jan 24]. <http://vietnamnet.vn/vn/suc-khoe/cac-loai-benh/cum-a-h5n1-la-gi-nhung-dac-diem-cua-cum-a-h5n1-382666.html>.

5. Heine HG, Foord AJ, Wang J, Vadeter S, Walker S, Morrissy C, et al. Detection of highly pathogenic zoonotic influenza virus H5N6 by reverse-transcriptase quantitative polymerase chain reaction. Virol J. 2015 Feb 8;12:18.

6. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. Universal primer set for the full-length amplification of all influenza A virus. Arch Virol. 2001 Dec;146(12):2275-89.

7. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA 6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013 Dec;30(12):2725-9. Epub 2013 Oct 16.

8. Environmental Systems Research Institute. ArcGIS. 2014 [cited 2018 Jan 24]. <http://resources.arcgis.com/en/help/main/10.2/index.html>.

9. R Project. Epicalc [cited 2018 Jan 24]. <https://cran.r-project.org/src/contrib/Archive/epicalc/>.

10. Vietnam. General Statistics Office of Vietnam. Ministry of Planning and Investment. Total poultry and cattle in Vietnam 2016. 2016 Oct 01. Vietnamese [cited 2018 Jan 24]. <http://www.gso.gov.vn/default.aspx?tabid=717>.

11. Vietnam Net. Consumption of 80 thousand tons of chicken from China. 2017 Feb 21. Vietnamese [cited 2018 Jan 24]. <http://www.vietnamnet.vn/vn/kinh-doanh/thi-truong/an-80-ngan-tan-ga-trung-quoc-thai-loai-am-anh-dich-benh-357386.html>.

12. Nguyen DT, Bryant JE, Davis CT, Nguyen LV, Pham LT, Loth L, et al. Prevalence and distribution of avian influenza a (H5N1) virus clade variants in live bird markets of Vietnam, 2011-2013. Avian Dis. 2014 Dec;58(4):599-608.

13. Liu J, Okazaki K, Ozaki H, Sakoda Y, Wu Q, Chen F, et al. H9N2 influenza viruses prevalent in poultry in China are phylogenetically distinct from A/quail/Hong Kong/G1/97 presumed to be the donor of the internal protein genes of the H5N1 Hong Kong/97 virus. Avian Pathol. 2003 Oct;32(5):551-60.

14. Bi Y, Chen Q, Wang Q, Chen J, Jin T, Wong G, et al. Genesis, evolution and prevalence of H5N6 avian influenza viruses in China. Cell Host Microbe. 2016 Dec 14;20(6):810-821. Epub 2016 Dec 1.

15. Chen H, Yuan H, Gao R, Zhang J, Wang D, Xiong Y, et al. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. Lancet. 2014 Feb 22;383(9918):714-21. Epub 2014 Feb 5.

16. Yang L1, Zhu W1, Li X1, Bo H1, Zhang Y1, Zou S1, et al. Genesis and dissemination of highly pathogenic H5N6 avian influenza viruses. J Virol. 2017 Feb 14;91(5). pii: e02199-16. Print 2017 Mar 1.