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Investigation of Highly Pathogenic Avian Influenza (H5N1) Outbreaks among Poultry in Ningxia, China, 2012

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Abstract

In April 2012, there were reports of suspected highly pathogenic avian influenza (HPAI) H5N1 among poultry layers in Ningxia. A retrospective investigation was conducted to describe epidemiological characteristics of the outbreak and identify risk factors for HPAI H5N1 infection. We administered a questionnaire to all 87 poultry layer farms in Touying County, Ningxia Province, within four weeks following the last reported outbreak. Samples of sick chickens were collected from 45 suspected farms to determine presence of H5N1 virus by reverse transcription polymerase chain reaction (RT-PCR) and confirmed by virus isolation. We used multiple logistic regression analysis to identify risk factors for infection and semivariogram analysis to demonstrate clustering of infected farms. Among 45 farms with positive PCR results, virus was isolated from four farms. Farms with improper waste disposal (Adjusted OR = 2.7, 95% CI = 1.20-8.30) and having visitors in farm within the past month (Adjusted OR = 5.5, 95% CI = 1.97-15.64) were significantly associated with HPAI infection. H5N1 was widespread in the county. Frequent human movement into farms and improper waste management increased the risk of infection. Biosecurity practices, including limiting number of visitors and proper waste disposal, should be enhanced to prevent further H5N1 infection.

Keywords: HPAI, H5N1, outbreak investigation, poultry layer

Introduction

The H5N1 subtype of highly pathogenic avian influenza (HPAI) infection is endemic in many countries around the world, including China. The first outbreak of HPAI H5N1 was reported in China on 27 Jan 2004. During the following months, 49 outbreaks were reported in 2004, resulting in culling of 9.02 million poultry.^{1,2}

HPAI H5N1 virus clades 7, 2.3.4 and 2.3.2 were the causes of most HPAI outbreaks in China since 2009.³

Compulsory vaccination combined with stamping out of infected flocks had been used to prevent and control HPAI since 2004.⁴ The H5N1 vaccines currently used in China, Re-4, Re-5 and Re-6, were derived from clades 7, 2.3.4 and 2.3.2 respectively.⁵

The H5N1 outbreaks declined dramatically, following mass vaccination in the country. However, H5N1 viruses can still be detected in live bird markets, causing sporadic outbreaks. Mutation frequency of H5N1 virus in China has increased because of

selection pressure caused by vaccination, antigenic shift and antigenic drift of H5N1 viruses.^{6,7}

The first outbreak of H5N1 in Ningxia Province was reported in 2006 and the viruses involved in that outbreak belonged to clade 7. Since then, Re-4 vaccine has been used to prevent H5N1 outbreaks in Ningxia. On 13 Apr 2012, several poultry farms in Touying County, Ningxia Province reported suspected outbreaks of HPAI to the local government. The outbreak was confirmed by the Ministry of Agriculture on 18 Apr 2012. Based on phylogenetic analysis of hemagglutinin (HA) gene, the virus involved in this outbreak was HPAI H5N1 virus clade 7.2 which shared 94.1% of HA sequence with Re-4 vaccine strain.⁸ A retrospective investigation was conducted in order to describe epidemiological characteristics of outbreak distribution in the affected county and identify risk factors associated with H5N1 infection.

Methods

Outbreak Investigation

We conducted an outbreak investigation in all 87 poultry layer farms within four weeks following the last report of an outbreak in Touying County, Ningxia during 2012. Trained investigators, consisting of provincial and local veterinarians, interviewed farmers using a structured questionnaire. The investigation team collected organs and cloacal swab samples from five sick chickens in each farm with suspected H5N1 infection. Presence of H5N1 virus was determined by reverse transcription polymerase

chain reaction (RT-PCR) and virus isolation. Isolated HPAI H5N1 viruses were confirmed by hemagglutination inhibition (HI) assay.

Suspected infected farms were layer farms in Touying County with poultry showing at least one of the following clinical signs: sudden death, drop in egg production, neurological signs, swollen head, hemorrhage on foot scale or cyanosis of comb, and positive RT-PCR result for H5N1 viruses. Confirmed farms were suspected farms with isolated H5N1 virus.

Geographic location of all poultry layer farms in Touying County was obtained from outside the farms using handheld global positioning system devices (UniStrong, Beijing) and analyzed by geographic information system, ArcGIS 10 (ESRI).

Risk Factor Analysis

A cross-sectional study was conducted using H5N1 suspected poultry farms as cases and all other non-infected poultry layer farms in Touying County as non-cases. All risk factors involved in the study were tested by chi-square or Fisher's exact test.

Biologically meaningful first-order interaction terms were also tested. Correlation between predictors was assessed using Pearson's correlation coefficient. Then, all factors with p-value less than 0.2 were considered for inclusion in a multivariable Bernoulli logistic regression model. All analyses were conducted using statistical software Stata version 11 (Stata corporation, College Station, Texas).

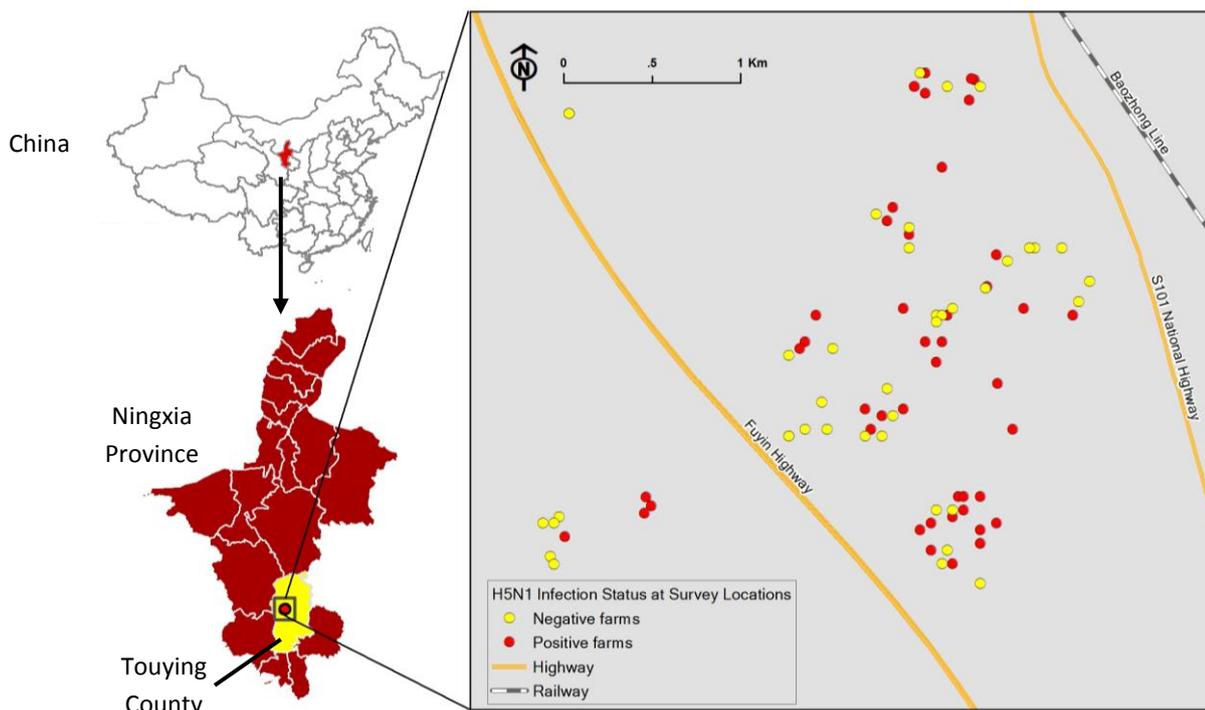


Figure 1. Geographical distribution of H5N1 farms in Ningxia Province, China, 2012

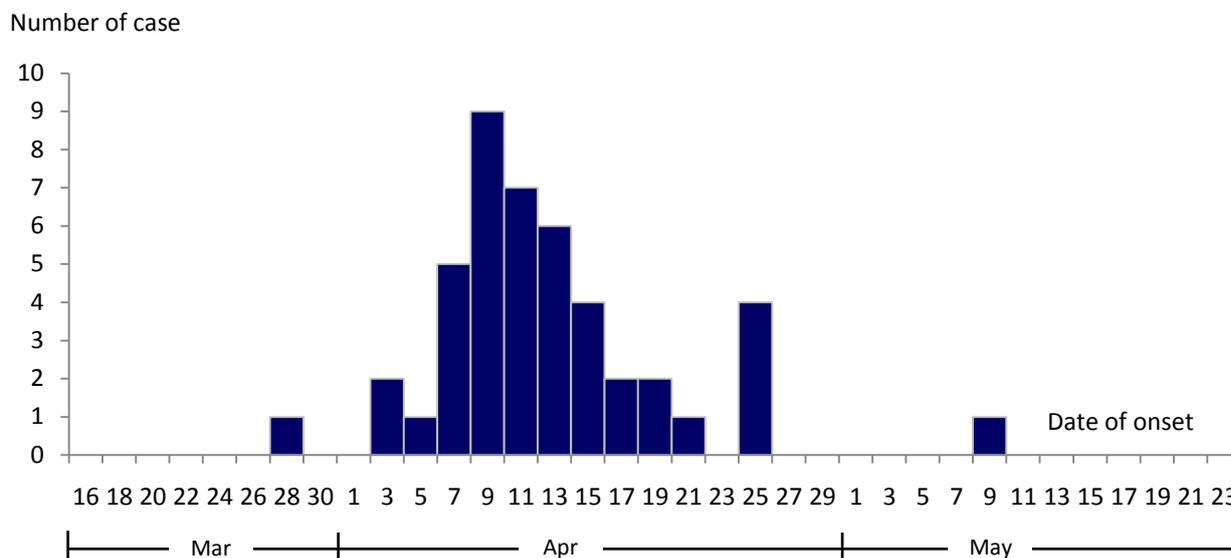


Figure 2. Epidemic curve of HPAI H5N1 outbreaks in 45 farms of Ningxia Province, China, 2012

Spatial Analysis

A semivariogram of residuals of final multivariable model (residual semivariogram) was used to quantify extent of spatial dependence left unaccounted by the variables in final multivariable model.⁹ Semivariogram is a graphical representation of a mathematical function which describes variability within a variable depending on location by examining the variation in observations with distance between all pairs of sampled locations. Residual semivariograms were constructed using geoR package in R version 2.10.0.

Results

Descriptive Epidemiology

All poultry farms in Touying County were poultry layer farms. Out of total 87 poultry layer farms in Touying County, 45 farms were suspected farms (51.7%). H5N1 viruses were isolated from four layer farms. Phylogenetic analysis revealed that the H5N1 viruses were belonged to clade 7.2.⁸ Geographical distribution of the 45 case farms and 42 non-case farms were shown in figure 1.

Clinical signs in the index farm began on 28 Mar 2012. There was a rapid increase in number of infected farms afterward. The number of newly suspected farms reached a peak on 9 Apr 2012, after which there was a reduction in number of suspected farms until 9 May 2012 when the last suspected farm was reported (Figure 2).

All infected flocks in the index farm were vaccinated at least once with Re-4 and Re-5 H5N1 vaccines, except in the first infected flock. The most common clinical signs in suspected farms were lethargy and

lack of appetite, drop in egg production and sudden death (Table 1). Mean proportion of mortality in all case farms was 17% (SD 3.0%). The highest mortality (83.3%) was found in non-vaccinated flock.

Table 1. Clinical signs of H5N1 outbreaks in 45 farms, Ningxia Province, China, 2012 (n=45)

Clinical sign	Infected farm	
	Number	Percent
Lethargy and lack of appetite	45	100.0
Drop in egg production	40	88.9
Sudden death	38	84.4
Cyanosis of comb	23	51.1
Neurological signs	18	40.0
Swollen head	6	13.3
Hemorrhage on foot scale	6	13.3

Factors Associated with HPAI H5N1 Infection

Results of the univariate analysis (Table 2) indicated that H5N1 infection was significantly associated with improper waste or dead bird disposal, no disinfection of egg tray and having visitors in farm. Improper waste or dead bird disposal were highly correlated ($r^2=0.9$).

Variables of improper waste disposal and having visitor in farm were retained in the final multivariable model. Results indicated that HPAI H5N1 case farms were almost three times more likely to have improper waste disposal (Adjusted OR = 2.7, 95% CI = 1.20-8.30) and five times more likely to have visitors prior to the outbreak (Adjusted OR = 5.5, 95% CI = 1.97-15.64) compared to non-case farms.

Table 2. Univariate analysis of factors associated with H5N1 outbreak in Ningxia Province, China, 2012

Factor	Category	Number of non-infected farm	Infected farm		Odds ratio (95% CI)	P-value
			Number	Percent		
Age of farm owner (year)	≥ 44	21	27	56.3	1.0 (0.96-1.08)	0.464
	< 44	21	18	46.2		
Farm as the main source of owners' income	Yes	39	40	50.6	1.6 (0.37-7.19)	0.396
	No	3	5	62.5		
Operating year	≥ 5	24	29	54.7	1.4 (0.57-3.22)	0.490
	< 5	18	16	47.1		
Breeding density (birds/m ²)	≥ 10	15	24	61.5	2.1 (0.87-4.87)	0.099
	< 10	27	21	43.8		
Improper waste disposal	Yes	19	9	32.1	3.3 (1.30-8.38)	0.012
	No	23	36	61.0		
Improper disposal of dead bird	Yes	19	11	36.7	2.6 (1.04-6.29)	0.041
	No	23	34	59.6		
Disinfection of egg tray	Yes	28	19	40.4	0.4 (0.15, 0.87)	0.022
	No	14	26	65.0		
Disinfection of external vehicle	Yes	15	10	40.0	0.5 (0.20, 1.32)	0.165
	No	27	35	56.5		
Introduction of new flocks	Yes	4	1	20.0	4.6 (0.59-36.15)	0.159
	No	38	44	53.7		
Having visitors in the farm	Yes	8	24	75.0	4.8 (1.85-12.78)	0.001
	No	34	21	38.2		
Workers visited to live bird market	Yes	2	3	60.0	1.4 (0.23-9.00)	0.533
	No	40	42	51.2		
Workers exposed to dead bird	Yes	5	8	61.5	1.6 (0.41-6.79)	0.443
	No	37	37	50.0		

Residual Spatial Variation in HPAI H5N1 Infection

We found that the variables included in the final multivariable model accounted for only 20% of clustering of infected farms (Figure 3). This suggested that location of farms in close proximity might explain the transmission of H5N1 in these farms.

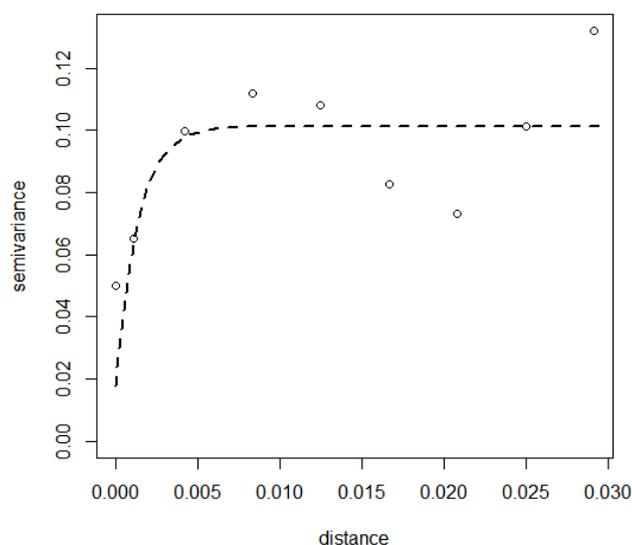


Figure 3. Semivariogram on residuals of the final multivariable model of HPAI H5N1 infection in Ningxia Province, China, 2012

Discussion

The outbreak of H5N1 HPAI in Touying County was confirmed by virus isolation. Four farms were proven to be H5N1 positive by virus isolation and 45 farms were H5N1 positive by RT-PCR. Recombinant events of H5N1 viruses with other avian influenza viruses had been reported in China in recent years.¹⁰⁻¹³ Some of the new variants have resulted as sporadic cases of HPAI in China. However, we did not find any new recombinant strains isolated from this outbreak. Continuous evolving of clade 7 viruses was found in another study conducted in the same area. Nucleotide sequence of viral HA gene only shared 94.1% of homology with that of A/chicken/Shanxi/2/2006 (H5N1), which was the prototype strain of clade 7.2 and the corresponding Re-4 vaccine.⁸ Some critical amino acid substitutions in viral HA1 domain occurred at antigenic sites resulted in variation between the field isolate and the vaccine strain. These substitutions such as K53E, K115E, S121H, E126N, A127T, G139E, K140N, T167A, D183N, K189M and T195N (H5 numbering), could change the antigenicity of the virus. In another study, the virus isolated was characterized by the HI assay using specific pathogen free (SPF) chicken serum against the vaccine strain prepared at China Animal Health and Epidemiology Center. HI titer of the vaccine strain was five folds more than the field isolate.⁸

These results indicated that the virus causing this outbreak was distinguishable from the vaccine strain. Chinese authorities also recognized the evolution of HPAI H5N1 virus clade 7 and considered a new vaccine candidate to use against this new emerging strain.

Early warning and rapid response is the key to reduce risk of transmission during an outbreak. Delayed reporting of suspected cases also play a role in rapid transmission of the disease. Thus, awareness of farmers on rapid reporting of suspected events should be enhanced. Timely reporting and investigations of die-off would enable animal health agents to record key information, concerning the first signs of an outbreak.

There might be some recall bias in the investigation since it was conducted retrospectively.

The findings in this study demonstrated that biosecurity practices could be important determinants for spread of HPAI H5N1. Susceptibility of poultry flocks to HPAI H5N1 infection could be reduced by implementation of vaccination policy. Once a farm has been confirmed as infected, immediate measures must be taken, including depopulation. In this study, high mortality was observed among non-vaccinated flocks of the index farm. Improper disposal of vaccinated flocks with asymptomatic infection of HPAI H5N1 virus in the same farm might spread the outbreak. A barrier must be created and maintained between farms and the outside world in order to prevent spread of a disease in and out of the farms. Visitors such as veterinarians or traders were also risk factors for transmission of H5N1 in this outbreak. Limiting number of people to contact with poultry could also prevent potential exposure of the virus to the poultry.

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Suggested Citation

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