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Risk Factors for Human Illness with Avian Influenza A (H5N1) Virus infection in China

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Abstract

Background—In China, 30 human cases of H5N1 virus infection have been identified to date. We conducted a retrospective case-control study to identify risk factors for H5N1 disease in China.

Methods—A questionnaire about potential H5N1 exposures was administered to 28 H5N1 cases and 134 randomly selected age, gender, and location matched controls or proxies. Conditional logistic regression analyses were performed.

Results—Before their illness, urban cases had visited wet poultry markets while rural cases had exposure to sick or dead backyard poultry. Independent H5N1 risk factors in multivariable analyses were direct contact with sick or dead poultry (OR 506.6; 95% CI 15.7–16319.6; p=0.0004), indirect

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Author Contributions

Hongjie Yu, Timothy Uyeki and Ray Y. Chen contributed to the study design; Lei Zhou, Qiaohong Liao, Libo Dong, and Yang Huai participated in the field investigations, collected data from participants for the study, and helped to analyze the data; Tian Bai, Yuelong Shu, Shiwen Wang, and Min Wang were responsible for supervising and performing the microneutralization and hemagglutinin inhibition assays, and analysis of serological data; Timothy Uyeki provided technical assistance for the epidemiological investigations and helped to review the data. Ray Y. Chen helped to review the data and contributed to revising the manuscript. All other co-authors participated in collection and management of data. Hongjie Yu supervised the overall study and wrote the protocol, was responsible for full access to all of the data, and takes responsibility for the integrity of the data and the accuracy of the data analysis. Hongjie Yu and Timothy Uyeki wrote the manuscript.

exposure to sick or dead poultry (OR 56.9; 95% CI 4.3–745.6; p=0.002), and visiting a wet poultry market (OR 15.4; 95% CI 3.0–80.2; p=0.001).

Conclusions—To prevent human H5N1 cases in China, education to avoid direct or close exposures to sick or dead poultry should be increased, and interventions to prevent the spread of H5N1 at live poultry markets should be implemented.

Keywords

H5N1; Risk Factors; China; Case-control Study

Introduction

In parallel with the unprecedented epizootic of highly pathogenic avian influenza (HPAI) A (H5N1) viruses among poultry and migratory birds [1], 387 confirmed human H5N1 cases with 245 deaths (as of October 20, 2008) had been reported from 15 countries since November 2003 [2]. Despite widespread exposures to H5N1 virus-infected poultry [3,4], human H5N1 disease remains rare to date and avian-to-human transmission of H5N1 virus is believed to have occurred in most human cases [5], with rare instances of limited, non-sustained human-to-human H5N1 virus transmission [6–8]. Environment-to-human transmission remains a possibility [5,9] for some human H5N1 cases without an identified exposure source. Although H5N1 virus has infected multiple animals [10,11], only poultry and wild birds have been implicated in transmission to humans to date.

Only limited data are available on risk factors associated with illness due to human infection with H5N1 viruses. A case-control study conducted during the 1997 H5N1 outbreak in Hong Kong Special Administrative Region (SAR), China, found that visiting a live poultry market the week before illness onset was the only significant H5N1 risk factor [12]. Studies conducted during 2004 in rural Thailand [13] and Vietnam [14] found that the most significant H5N1 risk factor was recent direct contact with sick or dead poultry.

Of the 30 confirmed human H5N1 cases reported to date in China, 29 were identified through surveillance from October 2005 [15] through July 2008 [2]. These 29 cases occurred sporadically and were distributed in 18 counties and 11 districts of 13 provinces with no obvious geographic clustering. One additional H5N1 case occurred in 2003 [16]. To inform prevention efforts, we conducted a retrospective matched case-control study to determine risk factors for human H5N1 illness in China.

Methods

Case-patients

In China, all suspected H5N1 cases are reported to the Chinese Center for Disease Control and Prevention (China CDC, Beijing, China) through a national surveillance system. A confirmed H5N1 case was defined as a patient with pneumonia or influenza-like illness (fever ≥38°C and cough or sore throat, with no other confirmed diagnosis), with laboratory evidence of H5N1 virus infection diagnosed by viral isolation or reverse transcriptase polymerase chain reaction (RT-PCR) by testing respiratory specimens, or a 4-fold or greater increase in H5N1 antibody titer in paired acute and convalescent sera. All 29 H5N1 cases identified by surveillance since 2005 were eligible to participate in the study. Exclusion criteria for cases included insufficient epidemiological data or inability to recruit matched controls. A rural case was defined as a village resident; an urban case was defined as a city resident.

Control Selection

Up to 5 randomly selected controls were matched to each case by gender, age (±1 year for cases <18 years old and ±5 years for adults ≥ 18 years old) and location. Eligible controls were persons who lived in the same location as the matched case for at least 3 months before the case's date of illness onset.

Two methods were utilized for random selection of potential controls. For rural cases, population registries from each case-patient's village were used to identify eligible age and gender-matched residents at the time of the case's symptom onset. Five potential controls were selected by using randomly generated numbers from the list of eligible controls. For urban cases, one apartment building immediately adjacent to the case's home was selected randomly. One floor in this building was selected randomly and all apartments on the floor were visited to recruit 5 controls. Additional controls were recruited from adjacent floors if needed. Inclusion criteria for eligible control participants were: absence of fever (temperature >37.5° C) or feverishness or respiratory illness during the 7 days before and after the matched case-patient's illness onset date, and testing seronegative for H5N1 antibodies.

Data collection

After trained investigators from the China CDC described the study's purpose to eligible cases and controls or their proxies and obtained written informed consent, participants were enrolled. A standardized questionnaire was used to collect information about demographic characteristics, underlying medical conditions, backyard poultry-raising, poultry H5 vaccination coverage levels, type of contact with sick/dead or well-appearing poultry, visits to places where live poultry were kept (e.g., wet poultry markets or poultry farms/factories), eating habits, exposure to other animals including wild birds, and exposure to other humans with acute respiratory illnesses or confirmed H5N1. Interviews were conducted a median of 360 days (range: 11–486) after matched cases' illness onset dates. A wet poultry market was defined as a place where small animals and poultry may be purchased live or slaughtered at the market [17]. Contact with sick/dead or well-appearing poultry was defined as direct contact (e.g. touching), and indirect contact - defined as no physical contact, but within 1 meter of poultry, poultry products, or poultry feces.

An adult household member (e.g., parent or legal guardian) closely familiar with the participants was interviewed as a proxy for any case-patient who died, was severely ill and unable to respond, or was aged <10 years old, and for controls aged <10 years old. For questions in which cases were asked about activities and exposures that occurred during the two weeks prior to their illness onset, controls were asked about the same activities and exposures during the same reference period.

Epidemiological and clinical data for 20 (71%) cases were collected previously during field investigations by China CDC staff as a public health response. These data were compared to the data collected from cases in our case-control study. Discrepancies were resolved in favor of the data obtained during the earlier field investigations.

If a proxy for any case or control was unable to provide sufficient information for the study, refused to participate, or no suitable proxy could be identified, the case or control was excluded from the study. Up to two visits were made in one week to recruit eligible persons to participate in the study. If selected controls were unavailable or declined to participate, the next eligible control was recruited to participate in the study.

Serological Testing

A single blood specimen was collected from surviving H5N1 case-patients and matched control participants at enrollment for H5N1 serological testing, which was performed at the National Influenza Center, China CDC by microneutralization assay [18] in a BSL-3 enhanced laboratory, and modified hemagglutinin-inhibition assay using horse red blood cells (horse HI) in BSL-2 conditions, as described previously [19]. Antigens for the assays were selected to match the genetic and antigenic characteristics of H5N1 virus strains that infected the matched human case if available, or were known to be circulating at the same times and locations where the cases occurred. Sera were tested in duplicate by two separate microneutralization assays conducted on different days. A serum specimen with an H5N1 neutralizing antibody titer of ≥ 1:80 was considered positive, with confirmation by horse HI [20,21]. Controls testing seropositive for H5N1 antibodies were excluded from the final analyses.

Statistical Analyses

Questionnaire data from cases and controls were entered in duplicate and verified using EpiData software (Odense, Denmark. Accessed at: http://www.epidata.dk/links.htm). Data were analyzed with SAS (version 9.13, SAS Institute Inc., Cary, NC, USA). Median and range values were calculated for continuous variables, and were compared between urban and rural cases using the Wilcoxon rank sum test. For categorical variables, frequencies for urban cases and rural cases were compared using Fisher's exact test. Baseline characteristics of cases and controls and independent associations between exposures and H5N1 disease were compared using exact conditional logistic regression. Matched odds ratios (OR) and 95% confidence intervals (CIs) were calculated for potential H5N1 risk factors. For multivariable exact conditional logistic analyses, we included variables with p 0.10 in univariate matched analyses for the initial model. Backward conditional logistic regression was performed by excluding variables with p>0.10. In matched analyses, if any case was missing exposure data, the data of all matching controls were excluded. However, if any control was missing exposure data, only the data from that control was excluded. All statistical tests were two-sided with a significance level of $\alpha = 0.05$.

Study Approval

The study protocol was approved by the Institutional Review Board of the China CDC. Written, signed, informed consent to participate in the study was obtained from adult participants or family member proxy for deceased cases. A parent or legal guardian provided written consent for participants aged <18 years, with participants aged 10–17 years also providing written informed assent.

Results

Twenty-eight (93%) H5N1 cases were enrolled in the study. We excluded two H5N1 cases occurring in military personnel with insufficient data: one from 2003 [16] and one from 2007 [22]. Of the 28 enrolled cases, H5N1 virus was isolated from 23 (82%), three (11%) were confirmed by RT-PCR and serology, and two (7%) were detected by serology only. All recruited controls agreed to participate and none withdrew from the study. Four cases (three rural, one urban) were matched to fewer than five controls due to unavailability of eligible controls. All controls tested seronegative for H5N1 antibodies. The final study population included 28 H5N1 cases and 134 matched controls. Data for cases (18 fatal, 1 severely ill and 3 cases aged <10 years old), were obtained by proxy interviews more often than controls (79% [n=22] *vs* 18% [n=24]). The baseline characteristics of case and control participants were similar for highest education level attained, annual household income, and smoking history (Table 1).

A descriptive analysis was performed to compare exposures between urban and rural cases (Table 2). Urban cases had a higher level of education, higher annual household income, and were significantly more likely to have visited a live poultry market than rural cases (100% [n=10] *vs* 39% [n=7], p=0.002). Rural cases were significantly more likely than urban cases to raise backyard poultry (83% [n=15] *vs* 0%, p=0.0001) or other animals (78% [n=14] *vs* 10% [n=1], p=0.001), have exposure to sick or dead poultry (78% [n=14] *vs* 10% [n=1], p=0.001), and to lack an indoor water supply (78% [n=14] *vs* 0%, p=0.0001). One urban case was exposed to a confirmed H5N1 case before illness onset [8]. One rural pediatric case was exposed to an ill sister with fever and respiratory illness 2 days before the case's illness onset [15].

In univariate analyses including all participants, the most significant risk factor was direct contact with sick or dead poultry (OR 34.7; 95% CI 4.3–276.9; p=0.001). Visiting a wet poultry market (OR 3.1; 95% CI 1.2–7.9; p=0.019) and having an underlying medical condition (OR 5.2; 95% CI 1.3–19.9; p=0.018) were also significant. Other significant risk factors are listed in Table 3. In univariate analyses restricted to rural participants, the most significant risk factors were direct contact with sick or dead poultry (OR 29.8; 95% CI 3.7–241.5; p=0.001) and only indirect contact (OR 11.3; 95% CI 2.2–58.5; p=0.004). Although a higher proportion of urban cases compared to controls visited a wet poultry market in the 2 weeks before illness onset (100% *vs.* 45%), these could not be compared statistically (Table 3).

Among the participants, five (18%) H5N1 cases and six (4%) controls had pertinent underlying medical conditions. Of the three female cases, all were adults, including two that were pregnant and one with a ten-year history of chronic bronchitis. Of the two male cases, one was a 15-year-old with a 10-year history of minimal change glomerulopathy requiring treatment at the time of illness onset, and a 24-year-old with Salmonella bacteremia identified at respiratory symptom onset and a history of intermittent fevers for the previous three months [8]. Of the six adult controls, four were pregnant, one female reported anemia, and one male had chronic bronchitis.

In multivariable analyses including all participants, significant independent H5N1 risk factors were direct contact with sick or dead poultry (OR 506.6; 95% CI 15.7–16319.6; p=0.0004), indirect exposure to sick or dead poultry (OR 56.9; 95% CI 4.3–745.6; p=0.002), and visiting a wet poultry market (OR 15.4; 95% CI 3.0–80.2; p=0.001). Direct contact (OR 67.3; 95% CI 5.8–783.8; p=0.0008) and indirect exposure to sick or dead poultry (OR 25.4; 95% CI 2.4–274.3; p=0.008) remained independent H5N1 risk factors when multivariable analyses were restricted to rural participants.

Discussion

We identified three independent risk factors for human H5N1 disease in China, including direct contact with sick or dead poultry, indirect exposure (<1 meter without direct contact) to sick/dead poultry, and visiting a wet poultry market. Direct contact with sick or dead poultry was the most significant H5N1 risk factor, consistent with previous studies [13,14]. Close indirect exposure to sick/dead poultry has also been reported in a descriptive study of Indonesian H5N1 cases [9]. This could reflect inhalation of aerosolized material contaminated with H5N1 viruses, or contact with surfaces or fomites contaminated with virus or with fertilizer containing fresh poultry feces, followed by self-inoculation of the respiratory tract [5], but our study design did not address this.

Our finding that visiting a wet poultry market in the two weeks before illness onset was a significant H5N1 risk factor is consistent with a case-control study conducted during the 1997 Hong Kong H5N1 outbreak [12]. Although widespread H5N1 poultry deaths were noted in wet markets during the Hong Kong outbreak, this has rarely been observed in urban China.

Wet poultry markets are considered a reservoir and amplifier of avian influenza A viruses because they bring together avian host species in a high-density setting that can facilitate viral persistence, cross-species infection and genetic reassortment [23,24]. H5N1 viral RNA was detected from an environmental specimen collected from a goose cage at a market that an urban H5N1 case had visited before onset [25] suggesting that H5N1 virus transmission via environmental contamination may occur in urban areas of China.

Most case-patients with H5N1 virus infection were previously healthy [5,26]. However, five (18%) of the 28 H5N1 cases had a pertinent underlying medical condition before illness and was a significant H5N1 risk factor in univariate analysis in our study. Although studies have shown that pregnant women and those with chronic pulmonary disease, renal dysfunction, hemoglobinopathies, or immunodeficiencies are at increased risk of complications from influenza [27], they may not necessarily be at increased risk of H5N1 virus infection. We were not able to analyze further the specific medical conditions in our study due to the small numbers but our data suggest that at least some of these conditions may be risk factors for H5N1 disease. Additional factors including pre-existing immunity or host genetic factors [28] might also contribute to the development of H5N1 disease, particularly for persons with underlying medical conditions. Further research is needed to understand the association between underlying medical conditions and H5N1 disease that we observed.

Chinese H5N1 cases comprised two distinct populations with respect to poultry exposures. Most rural Chinese raise backyard poultry for food production and income. In contrast, wet poultry markets are sustained by the demand for freshly slaughtered poultry in urban areas of China. Not surprisingly, exposures to poultry varied depending upon where the cases lived. Most urban cases had not exposed to sick or dead poultry or to backyard poultry before illness onset, but all had visited wet poultry markets, whereas most rural cases had exposure to backyard poultry and to sick or dead poultry. This suggests that public education and interventions to control disease should target different settings. Rural cases were less educated, poorer and more likely to lack an indoor water supply than urban cases, similar to a risk factor identified in Vietnam [14]. Because of the exposure differences between rural and urban cases, we performed analyses stratified by case location in addition to including all participants. The overall results were similar to the analyses restricted to rural participants alone.

Our study suggests that exposure to domestic waterfowl may be more of a risk to public health than contact with chickens. Studies in Vietnam, Thailand, and southern China have documented that domestic ducks and geese can be infected with HPAI H5N1 viruses without apparent symptoms [29–31]. Earlier studies conducted in 1997–2004 suggested that most H5N1 viral shedding in domestic ducks was in feces but, more recently, high H5N1 viral shedding has been detected in the upper respiratory tract of waterfowl for up to 17 days [31, 32]. Both respiratory and fecal shedding of H5N1 viruses can cause contamination of the environment and water sources used by birds and humans [5]. In univariate analyses, raising waterfowl such as ducks or geese was a risk factor for human H5N1 disease, but only raising backyard chickens was not. This suggests that domestically raised waterfowl exposure may pose a higher risk for avian-to-human transmission than exposure to backyard chickens in rural areas.

In China, a national H5 poultry vaccination program was implemented in 2005 [33], with documented decreases in poultry outbreaks [1]. However, the effectiveness of poultry H5 vaccination to reduce the risk of H5N1 virus transmission to humans is unknown. H5-vaccinated poultry that are infected with H5N1 viruses may shed fewer viruses or may not display clinical signs of disease, but could still be a risk to other poultry and to humans [34, 35]. Our findings suggest that very high H5 poultry vaccine coverage may be needed to reduce the risk of avian-to-human transmission of H5N1 viruses. Universal H5 poultry vaccination,

including domestic waterfowl, in conjunction with other control measures is recommended as an important control strategy by the World Animal Health Organization and the United Nations Food and Agriculture Organization [36]. The possibility that H5-vaccinated poultry may be infected with H5N1 viruses, but may not shed enough H5N1 viruses to transmit to humans was suggested by recent field evidence [37–39]. However, H5N1 cases continued to occur in China during 2006–2008 despite the national poultry H5 vaccination program. A simulation study showed that 'silent spread' of H5N1 can occur among poultry due to incomplete immunity at the flock level, even if a poultry vaccine is effective in individual birds [40]. Poultry H5 vaccine effectiveness studies are needed to examine outcomes such as H5N1 virus infection, as well as duration and quantitative viral shedding among vaccinated poultry to assess the public health risk, particularly in urban wet poultry markets.

There are a number of limitations to our findings. Since 20 (71%) case subjects and 98 (73%) matched controls were asked in 2007 about exposures that may have occurred much earlier, recall bias may have occurred if cases or their proxies were more likely to recall poultry exposures than controls. Although we interviewed H5N1 cases or their proxies long after the cases' illnesses occurred, nearly all of the case data collected in our study was concordant with data collected during the earlier field investigations. However, since no exposure data for controls was collected when cases occurred, the potential for differential recall and potential misclassification of some exposures could have introduced bias. A much higher proportion of cases' responses were provided by proxy interviews than in controls due to high mortality among cases, and these proxies may not have known all of the respective case's exposures. We could not verify the poultry H5 vaccination coverage reported by participants that raised backyard poultry. Although urban control participants were selected by a different method than rural controls, it is unlikely that selection bias was a significant limitation. All 28 cases had laboratory-confirmed H5N1 virus infection and all controls were seronegative for H5N1 neutralizing antibodies. Therefore, there was no misclassification of cases or controls on the basis of H5N1 virus infection status. A few variables that were collinear were included in the multivariable analysis, but did not influence the final results. Although our study included a higher number of participants than in previous case-control studies [12–14], the most important limitation was the small number of cases that precluded precise estimation of the magnitude of risk factors, and our study was underpowered to detect risk factors among urban H5N1 cases since nearly twice as many cases occurred in rural areas. Finally, it is possible that we did not identify all H5N1 cases that may have occurred in China during the study period.

Although human H5N1 disease is very rare, and persons with the risk factors we identified seldom develop H5N1 virus infection [41], interventions based upon our findings may help prevent further H5N1 virus transmission to humans in China. On-going education is needed that results in behavioral change to avoid direct or indirect contact with sick or dead poultry – which should be removed and disposed of promptly, utilizing appropriate protective equipment. In rural areas, ongoing efforts to achieve and maintain universal poultry H5 vaccination should be a high priority, especially among domestic waterfowl, and poultry should be raised outside the home. In urban areas, consideration should be given to implementing control strategies in wet poultry markets that have been instituted in Hong Kong SAR, such as only selling H5-vaccinated poultry, segregating bird species, improving biosecurity, having central poultry slaughtering locations, regular disinfection, and having a monthly rest day [42–44]. In addition, the feasibility of wearing protective masks or respirators for workers and visitors to wet poultry markets could be considered.

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Table 1

Baseline characteristics of H5N1 case-control study participants, China

Characteristics [a]	Cases (n=28)	Controls (n=134)	p-value
Median age (years, range)	29 (6–62)	29 (5–66)	–
Female (%)	15 (54)	74 (55)	–
Location			
Urban area (%)	10 (36)	49 (37)	–
Rural area (%)	18 (64)	85 (63)	–
Han ethnicity (%)	25 (89)	118 (88)	NA [c]
Interviewed by proxy (%)	22 (79)	24 (18)	NA [c]
Highest level of education			
Illiterate (%)	3 (11)	7 (5)	
Primary school (%)	8 (28)	50 (37)	
Junior high school (%)	9 (32)	40 (30)	0.485
High school (%)	5 (18)	19 (14)	
College or higher (%)	3 (11)	18 (14)	
Annual household income (RMB) [d]			
< 2000 (%)	9/27 (33)	42/129 (33)	
2000–4999 (%)	8/27 (30)	35/129 (27)	0.978
5000–10000 (%)	4/27 (15)	19/129 (15)	
> 10000 (%)	6/27 (22)	33/129 (25)	
Current smoker (%)	6 (21)	25 (19)	0.835
Seasonal influenza vaccination within past year (%)	0 (0)	2/131 (2)	NA [c]

[a] The denominators for calculation in fewer than the full groups were indicated.

[b] Comparison of frequencies between cases and controls were analyzed by exact conditional logistic regression. Matched factors (age, gender, location) were excluded in analyses. When p value was calculated, if any case was missing exposure data, the data of all matching controls were excluded. If any control was missing exposure data, only the data from that control was excluded.

[c] Not available, due to small sample size or data distribution not suitable for conditional logistic regression model.

[d] Exchange rate: $1 US = approximately 7.1 RMB.

Table 2

Demographic characteristics and exposures among 28 urban and rural human A (H5N1) cases, China

Characteristics [a]	Urban cases (n=10)	Rural cases (n=18)	p-value [b]
Age			
Median age (years, range)	30 (15–52)	25 (6–62)	0.443
6–14 years (%)	0 (0)	5 (28)	
15–59 years (%)	10 (100)	12 (67)	0.132
≥60 years (%)	0 (0)	1 (5)	
Female (%)	3 (30)	12 (67)	0.114
Highest level of education			
Illiterate (%)	0 (0)	3 (17)	
Primary school (%)	0 (0)	8 (44)	
Junior high school (%)	5 (50)	4 (22)	**0.006**
High school (%)	2 (20)	3 (17)	
College or higher (%)	3 (30)	0 (0)	
Annual household income (RMB) [c]			
< 2000 (%)	0 (0)	9/17 (53)	
2000–4999 (%)	1 (10)	7/17 (41)	**0.0001**
5000–10000 (%)	3 (30)	1/17 (6)	
> 10000 (%)	6 (60)	0/17 (0)	
Travel history [d] (%)	3 (30)	1 (6)	0.116
Occupational poultry exposure [e] (%)	1 (10)	3 (17)	1.000
Household with backyard poultry (%)	0 (0)	15 (83)	**0.0001**
Exposed to well-appearing poultry [f] (%)	10 (100)	17 (94)	1.000
Exposed to sick/dead poultry [g] (%)	1 (10)	14 (78)	**0.001**
Visited a wet poultry market (%)	10 (100)	7 (39)	**0.002**
Raised animals in home [h] (%)	1 (10)	14 (78)	**0.001**
Lack of indoor water supply (%)	0 (0)	14 (78)	**0.0001**
Exposed to persons with fever and respiratory symptoms (%)	0 (0)	1 (6) [i]	1.000
Exposed to confirmed human H5N1case-patients (%)	1 (10) [j]	0 (0)	0.357

[a] The denominators for calculation in fewer than the full groups were indicated.

[b] Comparison of frequencies between urban and rural cases were analyzed by Fisher's exact test, median age was compared with the Wilcoxon rank sum test.

[c] Exchange rate: 1 US $ = approximately 7.1 RMB.

[d] Travel outside of home-township (for rural cases) or outside of home city (for urban cases) for >24 hours in the 2 weeks prior to the case's illness onset.

[e] Defined as workplace exposure to live poultry (e.g., poultry farm/factory, wet poultry market), not including backyard poultry exposure.

[f] Includes direct and indirect contact with apparently well-poultry.

[g] Includes direct and indirect contact with sick/dead poultry.

[h] Includes cats, pigs, dogs, cows and goats.

[i] A family cluster was reported in reference 15.

[j] A family cluster consisting of confirmed son and his father was reported in reference 8.

Table 3

Univariate matched-pair analyses of potential H5N1 risk factors, overall and stratified by urban and rural groups, China

	Overall Participants				Rural Participants				Urban Participants			
Potential risk factors [a]	**Cases n=28, (%)**	**Controls n=134, (%)**	**OR (95% CI)**	**P** [b]	**Cases n=18, (%)**	**Controls n=85, (%)**	**OR (95% CI)**	**P** [b]	**Cases n=10, (%)**	**Controls n=49, (%)**	**OR (95% CI)**	**P** [b]
Underlying medicalcondition	5 (18)	6 (4)	5.2 (1.3–19.9)	**0.018**	3 (17)	4 (5)	5.6 (0.9–36.3)	**0.073**	2 (20)	2 (4)	4.7 (0.7–33.6)	**0.121**
Travel history [c]	4 (14)	20 (15)	1.0 (0.3–3.6)	0.964	1 (6)	13 (15)	0.2 (0.0–2.4)	0.208	3 (30)	7 (14)	2.8 (0.5–15.2)	0.222
Occupational poultry exposure [d]	4 (14)	5 (4)	13.1 (1.4–125.4)	**0.026**	3 (17)	5 (6)	8.3 (0.8–90.1)	**0.081**	1 (10)	0 (0)	NA [e]	NA [e]
Exposures to backyard poultry												
Raise backyard poultry	15 (54)	48 (36)	4.5 (1.1–17.5)	**0.031**	15 (83)	48 (56)	4.5 (1.1–17.5)	**0.031**	0 (0)	0 (0)	-	-
Location of backyard poultry cage												
No backyard poultry	13 (46)	86 (64)	ref [f]	ref [f]	3 (17)	37 (44)	ref [f]	ref [f]	-	-	-	-
Present outside home	9 (32)	37 (28)	3.7 (0.9–15.3)	**0.071**	9 (50)	37 (44)	3.7 (0.9–15.3)	**0.071**	-	-	-	-
Present inside house	6 (22)	11 (8)	9.7 (1.8–53.3)	**0.009**	6 (33)	11 (12)	9.7 (1.8–53.3)	**0.009**	-	-	-	-
Raise domestic waterfowl [g] or chickens												
No backyard poultry	13 (46)	86 (64)	ref [f]	ref [f]	3 (17)	37 (44)	ref [f]	ref [f]	-	-	-	-
Only raise chickens	7 (25)	34 (25)	2.6 (0.6–12.1)	0.226	7 (39)	34 (40)	2.6 (0.6–12.1)	0.226	-	-	-	-
Raise waterfowl	8 (29)	14 (11)	6.4 (1.6–26.3)	**0.010**	8 (44)	14 (16)	6.4 (1.6–26.3)	**0.010**	-	-	-	-
Backyard poultry H5 vaccination												
No backyard poultry	13 (46)	86/124 (70)	ref [f]	ref [f]	3 (17)	37/75 (50)	ref [f]	ref [f]	-	-	-	-
Backyard poultry												
H5 vaccination coverage ≥ 80%	6 (22)	19/124 (15)	4.0 (0.9–17.9)	**0.070**	6 (33)	19/75 (25)	4.0 (0.9–17.9)	**0.070**	-	-	-	-
Backyard poultry												
H5 vaccination coverage < 80%	9 (32)	19/124 (15)	7.1 (1.6–31.6)	**0.010**	9 (50)	19/75 (25)	7.1 (1.6–31.6)	**0.010**	-	-	-	-
Domestic waterfowl H5 vaccination												
No domestic waterfowl	20 (71)	120/132 (91)	ref [f]	ref [f]	10 (55)	71/83 (86)	ref [f]	ref [f]	-	-	-	-
Domestic waterfowl												
H5 vaccination coverage ≥ 80%	3 (11)	7/132 (5)	2.4 (0.5–11.2)	0.257	3 (17)	7/83 (8)	2.4 (0.5–11.2)	0.257	-	-	-	-
Domestic waterfowl												
H5 vaccination coverage < 80%	5 (18)	5/132 (4)	8.4 (1.6–45.1)	**0.013**	5 (28)	5/83 (6)	8.4 (1.6–45.1)	**0.013**	-	-	-	-
Exposures to well-appearing poultry												

Potential risk factors [a]	Overall Participants				Rural Participants				Urban Participants			
	Cases n=28, (%)	Controls n=134, (%)	OR (95% CI)	P [b]	Cases n=18, (%)	Controls n=85, (%)	OR (95% CI)	P [b]	Cases n=10, (%)	Controls n=49, (%)	OR (95% CI)	P [b]
Direct contact	11/27 (41)	31/133 (23)	3.3 (1.0–10.4)	**0.043**	9/17 (53)	27 (32)	2.9 (0.8–10.4)	**0.099**	2 (20)	4/48 (8)	5.3 (0.4–70.8)	0.206
Only indirect contact (<1 meter)	8/26 (31)	43/133 (32)	0.8 (0.3–2.4)	0.724	7/17 (41)	40/84 (48)	0.7 (0.2–2.3)	0.594	1/9 (11)	3 (6)	1.6 (0.1–19.4)	0.713
Consumed well-appearing poultry	22 (79)	99 (74)	1.3 (0.4–4.2)	0.610	12 (67)	59 (69)	0.8 (0.2–2.8)	0.689	10 (100)	40 (82)	NA [e]	NA [e]
Exposures to sick/dead poultry												
Direct contact with sick/dead poultry	9 (32)	4/133 (3)	34.7 (4.3–276.9)	**0.001**	8 (44)	4/84 (5)	29.8 (3.7–241.5)	**0.001**	1 (10)	0 (0)	NA [e]	NA [e]
Only indirect contact with sick/dead poultry (<1 meter)	6 (21)	4/132 (3)	11.3 (2.2–58.5)	**0.004**	6 (33)	4/83 (5)	11.3 (2.2–58.5)	**0.004**	-	-	-	-
Consumed sick/dead poultry	11 (39)	1 (1)	NA [e]	NA [e]	10 (56)	1 (1)	NA [e]	NA [e]	1 (10)	0 (0)	NA [e]	NA [e]
Wet poultry market exposures												
Visited wet poultry market	17 (61)	51/133 (38)	3.1 (1.2–7.9)	**0.019**	7 (39)	29/84 (35)	1.2 (0.4–3.8)	0.725	10 (100)	22 (45)	NA [e]	NA [e]
Visited wet poultry market and witnessed poultry slaughtering at market	15 (54)	35/129 (27)	5.0 (1.7–14.9)	**0.004**	6 (33)	17/83 (20)	2.2 (0.6–7.7)	0.224	9 (90)	18/46 (39)	NA [e]	NA [e]
Frequency of visiting wet poultry market within last 2 weeks												
Never (%)	11/27 (41)	82/131 (63)	ref [f]	ref [f]	11/17 (65)	55/82 (67)	ref [f]	ref [f]	0 (0)	27 (55)	ref [f]	ref [f]
1–5 times (%)	8/27 (30)	27/131 (20)	2.8 (0.9–8.1)	**0.062**	4/17 (23)	17/82 (21)	NA [e]	NA [e]	4 (40)	10 (21)	NA [e]	NA [e]
6–10 times (%)	3/27 (11)	8/131 (6)	7.6 (1.1–53.7)	**0.043**	2/17 (12)	2/82 (2)	NA [e]	NA [e]	1 (10)	6 (12)	NA [e]	NA [e]
>10 times (%)	5/27 (18)	14/131 (11)	5.8 (1.2–28.6)	**0.031**	0/17 (0)	8/82 (10)	NA [e]	NA [e]	5 (50)	6 (12)	NA [e]	NA [e]
Contact with live poultry in the market												
No contact	22/27 (82)	120/133 (90)	ref [f]	ref [f]	14/17 (82)	78/84 (92)	ref [f]	ref [f]	8 (80)	42 (86)	ref [f]	ref [f]
Only indirect contact with live poultry (<1 meter)	3/27 (11)	9/133 (7)	1.9 (0.4–8.1)	0.411	2/17 (12)	3/84 (4)	3.0 (0.5–19.2)	0.247	1 (10)	6 (12)	NA [e]	NA [e]
Direct contact with live poultry	2/27 (7)	4/133 (3)	4.6 (0.4–51.9)	0.222	1/17 (6)	3/84 (4)	2.4 (0.1–41.3)	0.534	1 (10)	1 (2)	NA [e]	NA [e]
Exposure to animals [h]												
Raise backyard animals	15 (54)	61 (46)	1.4 (0.6–3.7)	0.459	14 (78)	50 (59)	2.5 (0.7–8.9)	0.145	1 (10)	11 (22)	0.4 (0.0–3.2)	0.367
Direct contact with backyard animals	8 (29)	38 (28)	1.0 (0.4–2.6)	0.987	7 (39)	27 (32)	1.4 (0.5–4.0)	0.548	1 (10)	11 (22)	0.4 (0.0–3.4)	0.368
Lack of indoor water supply	14 (50)	68 (51)	0.7 (0.1–4.3)	0.726	14 (78)	68 (80)	0.7 (0.1–4.3)	0.726	0 (0)	0 (0)	-	-

Potential risk factors [a]	Overall Participants				Rural Participants				Urban Participants			
	Cases n=28, (%)	Controls n=134, (%)	OR (95% CI)	P [b]	Cases n=18, (%)	Controls n=85, (%)	OR (95% CI)	P [b]	Cases n=10, (%)	Controls n=49, (%)	OR (95% CI)	P [b]
Exposed to persons with fever and respiratory symptoms	1 (4) [l]	0 (0)	NA [e]	NA [e]	1 (6) [i]	0 (0)	NA [e]	NA [e]	0 (0)	0 (0)	-	-
Exposed to confirmed human H5N1 case-patients	1 (4) [j]	0 (0)	NA [e]	NA [e]	0 (0)	0 (0)	-	-	1 (10) [j]	0 (0)	NA [e]	NA [e]

[a] The denominators for calculation in fewer than the full groups were indicated.

[b] Comparison of frequencies between cases and controls were analyzed by exact conditional logistic regression. When matched OR and p value were calculated, data for matched controls were excluded for cases with missing exposure data, and controls with missing data were dropped from analyses, but not the matched case or other controls with available data.

[c] Travel outside of home-township (for rural cases) or outside of home city (for urban cases) for >24 hours in the 2 weeks prior to the case's illness onset.

[d] Defined as workplace exposure to live poultry (e.g., poultry farm/factory, wet poultry market), not including backyard poultry exposure

[e] Not available, due to small sample size or data distribution could not be analyzed by conditional logistic regression

[f] ref: reference.

[g] Includes ducks and geese.

[h] Includes cats, pigs, dogs, cows and goats.

[i] A family cluster was reported in reference 15.

[j] A family cluster consisting of a confirmed son and his father was reported in reference 8.