MAJOR ARTICLE

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

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(See the article by Tiensin et al. on pages 1735–43, the article by Vong et al. on pages 1744–52, and the editorial commentary by Briand and Fukuda on pages 1717–9)

Background. In China, 30 human cases of avian influenza A (H5N1) virus infection were identified through July 2008. We conducted a retrospective case-control study to identify risk factors for influenza H5N1 disease in China.

Methods. A questionnaire about potential influenza H5N1 exposures was administered to 28 patients with influenza H5N1 and to 134 randomly selected control subjects matched by age, sex, and location or to proxies. Conditional logistic regression analyses were performed.

Results. Before their illness, patients living in urban areas had visited wet poultry markets, and patients living in rural areas had exposure to sick or dead backyard poultry. In multivariable analyses, independent risk factors for influenza H5N1 were direct contact with sick or dead poultry (odds ratio [OR], 506.6 [95% confidence interval {CI}, 15.7–16319.6]; $P < .001$), indirect exposure to sick or dead poultry (OR, 56.9 [95% CI, 4.3–745.6]; $P = .002$), and visiting a wet poultry market (OR, 15.4 [95% CI, 3.0–80.2]; $P = .001$).

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

In parallel with the unprecedented epizootic of highly pathogenic avian influenza A (H5N1) viruses among poultry and migratory birds [1], 418 confirmed human cases of influenza H5N1 with 257 deaths were reported in 15 countries from November 2003 through 17 April 2009 [2]. Despite widespread human exposure to influenza H5N1 virus–infected poultry [3, 4], human influenza H5N1 disease remains rare, and avian-to-human transmission of influenza H5N1 virus is believed to have occurred in most human cases [5], with rare instances of limited, nonsustained human-to-human influenza H5N1 virus transmission [6–8]. Environment-to-human transmission remains a possibility [5, 9] for some human influenza H5N1 cases without an identified exposure source. Although influenza H5N1 virus has infected multiple species of animals [10, 11], to date, only poultry and wild birds have been implicated in transmission to humans.

Only limited data are available on risk factors associated with illness caused by human infection with influenza H5N1 viruses. A case-control study conducted during the 1997 outbreak of influenza H5N1 in Hong Kong Special Administrative Region, China, found that hav-

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ing visited a live poultry market the week before illness onset was the only significant risk factor for influenza H5N1 [12]. Studies conducted during 2004 in rural Thailand [13] and Vietnam [14] found that the most significant risk factor for influenza H5N1 was recent direct contact with sick or dead poultry.

Of the 38 confirmed human influenza H5N1 cases reported to date in China, 30 had occurred as of July 2008. Of these, 29 were identified through surveillance from October 2005 [15] through July 2008 [2]. These 29 cases occurred sporadically and were distributed across 18 counties and 11 districts of 13 provinces, with no obvious geographic clustering. One additional influenza H5N1 case occurred during 2003 [16]. To inform prevention efforts, we conducted a retrospective matched case-control study to determine risk factors for human influenza H5N1 illness in China.

SUBJECTS AND METHODS

Patients. In China, all suspected influenza H5N1 cases are reported to the Chinese Center for Disease Control and Prevention (China CDC) through a national surveillance system. A confirmed case of influenza H5N1 was defined as pneumonia or influenza-like illness (marked by fever [temperature, $\geq$38°C] and cough or sore throat, with no other confirmed diagnosis), with laboratory evidence of influenza H5N1 virus infection with use of viral isolation or reverse-transcription polymerase chain reaction of respiratory specimens or with a $\geq$4-fold increase in influenza H5N1 antibody titer in paired acute- and convalescent-phase serum samples. All 29 patients with influenza H5N1 who were identified by surveillance from October 2005 through July 2008 were eligible to participate in the study. Exclusion criteria for patients included insufficient epidemiological data or inability to recruit matched control subjects. A rural patient was defined as a village resident, and an urban patient was defined as a city resident.

Control subject selection. Up to 5 randomly selected control subjects were matched with each patient by sex, age ($\pm$1 year for patients aged $<$18 years and $\pm$5 years for patients aged $\geq$18 years), and location. Eligible control subjects were persons who lived in the same location as the matched patient for at least 3 months before the date of illness onset in the patient.

Two methods were used for random selection of potential control subjects. For rural patients, population registries from each patient's village were used to identify eligible age- and sex-matched residents at the time of symptom onset in the patient. Five potential control subjects were selected using randomly generated numbers from the list of eligible control subjects. For urban patients, 1 apartment building immediately adjacent to the patient's home was selected randomly. One floor in this building was selected randomly, and all apartments on the floor were visited to recruit 5 control subjects. Additional control subjects were recruited from adjacent floors if needed. Inclusion criteria for eligible control subjects were absence of fever (temperature, $>$37.5°C), feverishness, and respiratory illness during the 7 days before and after the matched patient's illness onset date and having a specimen test seronegative for influenza H5N1 antibodies.

Data collection. After trained investigators from the China CDC described the purpose of the study to eligible patients and control subjects 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 and/or dead or healthy-appearing poultry, visits to places where live poultry were kept (e.g., wet poultry markets or poultry farms and/or factories), eating habits, exposure to other animals (including wild birds), and exposure to other humans with acute respiratory illnesses or confirmed influenza H5N1. Interviews were conducted a median of 360 days (range, 11–486 days) after the date of onset of illness in the patient. A wet poultry market was defined as a place where small animals and poultry may be purchased live or slaughtered [17]. Contact with sick and/or dead or healthy-appearing poultry was defined as direct contact (e.g., touching) and indirect contact (defined as no physical contact but being within 1 m of poultry, poultry products, or poultry feces).

An adult household member (e.g., parent or legal guardian) who was closely familiar with the participants was interviewed as a proxy for any patient who died, was severely ill and unable to respond, or was aged $<$10 years and for control subjects aged $<$10 years. For questions posed to patients about activities and exposures that occurred during the 2 weeks before their illness onset, control subjects were asked about the same activities and exposures during the same reference period.

Epidemiological and clinical data for 20 (71%) of the 28 patients enrolled in the study were previously collected during field investigations by China CDC staff as a public health response. These data were compared with the data collected from patients in our case-control study. Discrepancies were resolved in favor of the data obtained during the earlier field investigations.

If a proxy for any patient or control subject was unable to provide sufficient information for the study or refused to participate or if no suitable proxy could be identified, the patient or control subject was excluded from the study. Up to 2 visits were made in 1 week to recruit eligible persons to participate in the study. If selected control subjects were unavailable or declined participation, the next eligible control subject was recruited to participate in the study.

Serological testing. A single blood specimen was collected from surviving patients with influenza H5N1 and from matched control subjects at enrollment for influenza H5N1 serological testing, which was performed at the National Influenza Center (China CDC; Beijing) with use of a microneutralization assay

[18] in a biosafety level 3 enhanced laboratory and with use of a modified hemagglutinin-inhibition assay with horse red blood cells under biosafety level 2 conditions, as described elsewhere [19]. Antigens for the assays were selected to match the genetic and antigenic characteristics of the influenza H5N1 virus strains that infected the matched patients, if available, or that were known to be circulating at the same times when and locations where the cases occurred. Serum samples were tested in duplicate by 2 separate microneutralization assays conducted on different days. A serum specimen with an influenza H5N1 neutralizing antibody titer of $\geqslant$1:80 was considered to be positive, with confirmation by the hemagglutinin-inhibition assay with horse red blood cells [20, 21]. Control subjects whose specimens tested seropositive for influenza H5N1 antibodies were excluded from the final analyses.

Statistical analyses. Questionnaire data from patients and control subjects were entered in duplicate and were verified using EpiData software. Data were analyzed using SAS, version 9.13 (SAS Institute). Median and range values were calculated for continuous variables and were compared between urban and rural patients with use of the Wilcoxon rank sum test. For categorical variables, frequencies of urban cases and rural cases were compared using Fisher's exact test. Baseline characteristics of patients and control subjects and independent associations between exposures and influenza H5N1 disease were compared using exact conditional logistic regression. Matched odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for potential influenza H5N1 risk factors. For multivariable exact conditional logistic analyses, we included variables with $P \leqslant .10$ in univariate matched analyses for the initial model. Backward conditional logistic regression was performed by excluding variables with $P > .10$. In matched analyses, if any patient was missing exposure data, the data for all matching control subjects were excluded. However, if any control subject was missing exposure data, only the data for that control subject were excluded. All statistical tests were 2-sided, with a significance level of $\alpha = .05$.

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

RESULTS

Twenty-eight patients (97%) with influenza H5N1 were enrolled in the study. We excluded 1 case of influenza H5N1 that occurred in military personnel with insufficient data: 1 from 2003 [16] and 1 from 2007 [22]. Among the 28 enrolled patients, influenza H5N1 virus was detected by isolation for 23 (82%), by reverse-transcriptase polymerase chain reaction and serologic testing for 3 (11%), and by serologic testing only for 2 (7%). All recruited control subjects agreed to participate, and none withdrew from the study. Four patients (3 rural and 1 urban) were matched to <5 control subjects because of unavailability of eligible control subjects. Samples from all control subjects tested seronegative for influenza H5N1 antibodies. The final study population included 28 patients with influenza H5N1 and 134 matched control subjects. Data for patients (18 died, 1 was severely ill, and 3 were aged <10 years) were obtained by proxy interviews more often than were data for control subjects (22 [79%] vs. 24 [18%]). The baseline characteristics of patients and control subjects were similar for highest education level attained, annual household income, and smoking history (table 1).

Table 1. Baseline characteristics of participants in a case-control study of influenza H5N1 in China.

Characteristic	Patients (n = 28)	Control subjects (n = 134)	P[a]
Age, median (range), years	29 (6–62)	29 (5–66)	
Female sex	15 (54)	74 (55)	
Location			
Urban area	10 (36)	49 (37)	
Rural area	18 (64)	85 (63)	
Han ethnicity	25 (89)	118 (88)	NA
Interviewed by proxy	22 (79)	24 (18)	NA
Highest level of education			
Illiterate	3 (11)	7 (5)	.485
Primary school	8 (29)	50 (37)	
Junior high school	9 (32)	40 (30)	
High school	5 (18)	19 (14)	
College or higher	3 (11)	18 (13)	
Annual household income, RMB[b]			
<2000	9 (33)	42 (33)	.978
2000–4999	8 (30)	35 (27)	
5000–10,000	4 (15)	19 (15)	
>10,000	6 (22)	33 (26)	
Current smoker	6 (21)	25 (19)	.835
Seasonal influenza vaccination within past year	0 (0)	2 (2)[c]	NA

NOTE. Data are no. (%) of participants, unless otherwise indicated. NA, not available (because of small sample size or because data distribution was not suitable for conditional logistic regression model).

[a] Comparison of frequencies between patients and control subjects were analyzed by exact conditional logistic regression. Matched factors (age, sex, and location) were excluded from analyses. When the P value was calculated, if any patient was missing exposure data, the data of all matching control subjects were excluded. If any control subject was missing exposure data, only the data from that control subject were excluded.

[b] Data are for 27 patients and 129 control subjects. Exchange rate: US$1 is equal to ~7.1 RMB.

[c] Data are for 131 control subjects.

A descriptive analysis was performed to compare exposures between urban and rural patients (table 2). Urban patients (n = 10) had a higher level of education and a higher annual household income and were significantly more likely to have

Table 2. Demographic characteristics and exposures of 28 urban and rural patients with human influenza A (H5N1) in China.

Characteristic	Urban patients (n = 10)	Rural patients (n = 18)	P[a]
Age, years			
Median (range)	30 (15–52)	25 (6–62)	.443
6–14	0 (0)	5 (28)	.132
15–59	10 (100)	12 (67)	
⩾60	0 (0)	1 (5)	
Female sex	3 (30)	12 (67)	.114
Highest level of education			
Illiterate	0 (0)	3 (17)	**.006**
Primary school	0 (0)	8 (44)	
Junior high school	5 (50)	4 (22)	
High school	2 (20)	3 (17)	
College or higher	3 (30)	0 (0)	
Annual household income, RMB[b]			
<2000	0 (0)	9 (53)	**<.001**
2000–4999	1 (10)	7 (41)	
5000–10,000	3 (30)	1 (6)	
>10,000	6 (60)	0 (0)	
Travel history[c]	3 (30)	1 (6)	.116
Occupational poultry exposure[d]	1 (10)	3 (17)	>.99
Household with backyard poultry	0 (0)	15 (83)	**<.001**
Exposure to healthy-appearing poultry[e]	10 (100)	17 (94)	>.99
Exposure to sick and/or dead poultry[f]	1 (10)	14 (78)	**.001**
Visited a wet poultry market	10 (100)	7 (39)	**.002**
Raised animals in home[g]	1 (10)	14 (78)	**.001**
Lack of indoor water supply	0 (0)	14 (78)	**<.001**
Exposed to persons with fever and respiratory symptoms	0 (0)	1 (6)[h]	>.99
Exposed to a person with confirmed influenza H5N1	1 (10)[i]	0 (0)	.357

NOTE. Data are no. (%) of patients. Boldface indicates statistical significance.

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

[b] Data are for 17 control subjects. Exchange rate: US$1 is equal to ~7.1 RMB.

[c] Travel outside home township (for rural patients) or outside home city (for urban patients) for >24 h during the 2 weeks prior to the patient's illness onset.

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

[e] Includes direct and indirect contact with apparently healthy poultry.

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

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

[h] A family cluster was reported in Yu et al. [15].

[i] A family cluster consisting of confirmed son and his father was reported in Wang et al. [8].

visited a live poultry market, compared with rural patients (n = 18; 10 [100%] vs. 7 [39%]; P = .002). Rural patients were significantly more likely than urban patients to raise backyard poultry (15 [83%] vs. 0 [0%]; P < .001) or other animals (14 [78%] vs. 1 [10%]; P = .001), to have had exposure to sick or dead poultry (14 [78%] vs. 1 [10%]; P = .001), and to lack an indoor water supply (14 [78%] vs. 0 [0%]; P = .001). One urban patient was exposed to a person with confirmed influenza H5N1 before illness onset [8]. One rural pediatric patient was exposed to an ill sister with fever and respiratory illness 2 days before 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 = .001). Visiting a wet poultry market (OR, 3.1 [95% CI, 1.2–7.9]; P = .019) and having an underlying medical condition (OR, 5.2 [95% CI, 1.3–19.9]; P = .018) were also statistically 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 = .001) and indirect contact only (OR, 11.3 [95% CI, 2.2–58.5]; P = .004). Although a higher proportion of urban patients than control subjects visited a wet poultry market during the 2 weeks before illness onset (100% vs. 45%), these proportions could not be compared statistically (table 3).

Among the participants, 5 patients (18%) and 6 control subjects (4%) had pertinent underlying medical conditions. Of the 3 female patients, all were adults, including 2 who were pregnant and 1 who had a 10-year history of chronic bronchitis. Of the 2 male patients, 1 was aged 15 years and had a 10-year history of minimal-change glomerulopathy that required treatment at the time of illness onset, and 1 was aged 24 years, had *Salmonella* bacteremia identified at the time of onset of respiratory symptoms, and had intermittent fevers during the previous 3 months [8]. Of the 6 adult control subjects, 4 were pregnant women, 1 was a woman who reported anemia, and 1 was a man with 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 < .001), indirect exposure to sick or dead poultry (OR, 56.9 [95% CI, 4.3–745.6]; P = .002), and visiting a wet poultry market (OR, 15.4 [95% CI, 3.0–80.2]; P = .001). Direct contact (OR, 67.3 [95% CI, 5.8–783.8]; P < .001) and indirect exposure to sick or dead poultry (OR, 25.4 [95% CI, 2.4–274.3]; P = .008) remained independent risk factors for influenza H5N1 when multivariable analyses were restricted to rural participants.

DISCUSSION

We identified 3 independent risk factors for human influenza H5N1 disease in China, including direct contact with sick or dead poultry, indirect exposure (being within 1 m without direct contact) to sick and/or dead poultry, and visiting a wet poultry market. Direct contact with sick or dead poultry was the most

Table 3. Univariate matched-pair analyses of potential risk factors for influenza H5N1, overall and stratified by urban and rural groups, in China.

	Participants											
	All				Rural				Urban			
Potential risk factor	Patients (*n*= 28)	Control subjects (*n* = 134)	OR (95% CI)	*P*[a]	Patients (*n* = 18)	Control subjects (*n* = 85)	OR (95% CI)	*P*[a]	Patients (*n* = 10)	Control subjects (*n* = 49)	OR (95% CI)	*P*[a]
Underlying medical condition	5/28 (18)	6/134 (4)	5.2 (1.3–19.9)	**.018**	3/18 (17)	4/85 (5)	5.6 (0.9–36.3)	**.073**	2/10 (20)	2/49 (4)	4.7 (0.7–33.6)	**.121**
Travel history[b]	4/28 (14)	20/134 (15)	1.0 (0.3–3.6)	.964	1/18 (6)	13/85 (15)	0.2 (0.0–2.4)	.208	3/10 (30)	7/49 (14)	2.8 (0.5–15.2)	.222
Occupational poultry exposure[c]	4/28 (14)	5/134 (4)	13.1 (1.4–125.4)	**.026**	3/18 (17)	5/85 (6)	8.3 (0.8–90.1)	**.081**	1/10 (10)	0/49 (0)	NA	
Raise backyard poultry	15/28 (54)	48/134 (36)	4.5 (1.1–17.5)	**.031**	15/18 (83)	48/85 (56)	4.5 (1.1–17.5)	**.031**	0/10 (0)	0/49 (0)	. . .	
Location of backyard poultry cage												
No backyard poultry	13/28 (46)	86/134 (64)	Ref		3/18 (17)	37/85 (44)	Ref		. . .	. . .	. . .	
Present outside house	9/28 (32)	37/134 (28)	3.7 (0.9–15.3)	**.071**	9/18 (50)	37/85 (44)	3.7 (0.9–15.3)	**.071**	. . .	. . .	. . .	
Present inside house	6/28 (22)	11/134 (8)	9.7 (1.8–53.3)	**.009**	6/18 (33)	11/85 (12)	9.7 (1.8–53.3)	**.009**	. . .	. . .	. . .	
Raise domestic waterfowl[d] or chickens												
No backyard poultry	13/28 (46)	86/134 (64)	Ref		3/18 (17)	37/85 (44)	Ref		. . .	. . .	. . .	
Only raise chickens	7/28 (25)	34/134 (25)	2.6 (0.6–12.1)	.226	7/18 (39)	34/85 (40)	2.6 (0.6–12.1)	.226	. . .	. . .	. . .	
Raise waterfowl	8/28 (29)	14/134 (11)	6.4 (1.6–26.3)	**.010**	8/18 (44)	14/85 (16)	6.4 (1.6–26.3)	**.010**	. . .	. . .	. . .	
Backyard poultry H5 vaccination												
No backyard poultry	13/28 (46)	86/124 (70)	Ref		3/18 (17)	37/75 (50)	Ref		. . .	. . .	. . .	
Vaccination coverage ⩾80%	6/28 (22)	19/124 (15)	4.0 (0.9–17.9)	**.070**	6/18 (33)	19/75 (25)	4.0 (0.9–17.9)	**.070**	. . .	. . .	. . .	
Vaccination coverage <80%	9/28 (32)	19/124 (15)	7.1 (1.6–31.6)	**.010**	9/18 (50)	19/75 (25)	7.1 (1.6–31.6)	**.010**	. . .	. . .	. . .	
Domestic waterfowl H5 vaccination												
No domestic waterfowl	20/28 (71)	120/132 (91)	Ref		10/18 (55)	71/83 (86)	Ref		. . .	. . .	. . .	
Vaccination coverage ⩾80%	3/28 (11)	7/132 (5)	2.4 (0.5–11.2)	.257	3/18 (17)	7/83 (8)	2.4 (0.5–11.2)	.257	. . .	. . .	. . .	
Vaccination coverage <80%	5/28 (18)	5/132 (4)	8.4 (1.6–45.1)	**.013**	5/18 (28)	5/83 (6)	8.4 (1.6–45.1)	**.013**	. . .	. . .	. . .	
Exposures to healthy-appearing poultry												
Direct contact	11/27 (41)	31/133 (23)	3.3 (1.0–10.4)	**.043**	9/17 (53)	27/85 (32)	2.9 (0.8–10.4)	**.099**	2/10 (20)	4/48 (8)	5.3 (0.4–70.8)	.206
Only indirect contact (within 1 m)	8/26 (31)	43/133 (32)	0.8 (0.3–2.4)	.724	7/17 (41)	40/84 (48)	0.7 (0.2–2.3)	.594	1/9 (11)	3/49 (6)	1.6 (0.1–19.4)	.713
Consumed healthy-appearing poultry	22/28 (79)	99/134 (74)	1.3 (0.4–4.2)	.610	12/18 (67)	59/85 (69)	0.8 (0.2–2.8)	.689	10/10 (100)	40/49 (82)	NA	
Exposures to sick and/or dead poultry												
Direct contact	9/28 (32)	4/133 (3)	34.7 (4.3–276.9)	**.001**	8/18 (44)	4/84 (5)	29.8 (3.7–241.5)	**.001**	1/10 (10)	0/49 (0)	NA	
Only indirect contact (within 1 m)	6/28 (21)	4/132 (3)	11.3 (2.2–58.5)	**.004**	6/18 (33)	4/83 (5)	11.3 (2.2–58.5)	**.004**	. . .	. . .	. . .	
Consumed	11/28 (39)	1/134 (1)	NA		10/18 (56)	1/85 (1)	NA		1/10 (10)	0/49 (0)	NA	
Wet poultry market exposure												
Visited wet poultry market	17/28 (61)	51/133 (38)	3.1 (1.2–7.9)	**.019**	7/18 (39)	29/84 (35)	1.2 (0.4–3.8)	.725	10/10 (100)	22/49 (45)	NA	
Visited wet poultry market and witnessed poultry slaughtering at market	15/28 (54)	35/129 (27)	5.0 (1.7–14.9)	**.004**	6/18 (33)	17/83 (20)	2.2 (0.6–7.7)	.224	9/10 (90)	18/46 (39)	NA	

Frequency of visits to wet poultry market within 2 weeks before illness onset												
Never	11/27 (41)	82/131 (63)	Ref		11/17 (65)	55/82 (67)	Ref		0/10 (0)	27/49 (55)	Ref	
1–5 times	8/27 (30)	27/131 (20)	2.8 (0.9–8.1)	**.062**	4/17 (23)	17/82 (21)	NA		4/10 (40)	10/49 (21)	NA	
6–10 times	3/27 (11)	8/131 (6)	7.6 (1.1–53.7)	**.043**	2/17 (12)	2/82 (2)	NA		1/10 (10)	6/49 (12)	NA	
>10 times	5/27 (18)	14/131 (11)	5.8 (1.2–28.6)	**.031**	0/17 (0)	8/82 (10)	NA		5/10 (50)	6/49 (12)	NA	
Contact with live poultry at the market												
No contact	22/27 (82)	120/133 (90)	Ref		14/17 (82)	78/84 (92)	Ref		8/10 (80)	42/49 (86)	Ref	
Only indirect contact (within 1 m)	3/27 (11)	9/133 (7)	1.9 (0.4–8.1)	.411	2/17 (12)	3/84 (4)	3.0 (0.5–19.2)	.247	1/10 (10)	6/49 (12)	NA	
Direct contact	2/27 (7)	4/133 (3)	4.6 (0.4–51.9)	.222	1/17 (6)	3/84 (4)	2.4 (0.1–41.3)	.534	1/10 (10)	1/49 (2)	NA	
Exposure to animals [e]												
Raise backyard animals	15/28 (54)	61/134 (46)	1.4 (0.6–3.7)	.459	14/18 (78)	50/85 (59)	2.5 (0.7–8.9)	.145	1/10 (10)	11/49 (22)	0.4 (0.0–3.2)	.367
Direct contact with backyard animals	8/28 (29)	38/134 (28)	1.0 (0.4–2.6)	.987	7/18 (39)	27/85 (32)	1.4 (0.5–4.0)	.548	1/10 (10)	11/49 (22)	0.4 (0.0–3.4)	.368
Lack of indoor water supply	14/28 (50)	68/134 (51)	0.7 (0.1–4.3)	.726	14/18 (78)	68/85 (80)	0.7 (0.1–4.3)	.726	0/10 (0)	0/49 (0)	. . .	
Exposed to persons with fever and respiratory symptoms	1/28 (4)[f]	0/134 (0)	. . .		1/18 (6)[f]	0/85 (0)	NA		0/10 (0)	0/49 (0)	. . .	
Exposed to persons with confirmed influenza H5N1	1/28 (4) [g]	0/134 (0)	. . .		0/18 (0)	0/85 (0)	. . .		1/10 (10)[g]	0/49 (0)	NA	

NOTE. Data are proportion (%) of participants, unless otherwise indicated. Boldface indicates $P \leq .10$, and those variables with $P \leq .10$ were included in univariate matched analyses for the initial model. CI, confidence interval; NA, not available (because of small sample size or because data distribution could not be analyzed by conditional logistic regression); OR, odds ratio; Ref, reference.

[a] Comparison of frequencies between patients and control subjects were analyzed by exact conditional logistic regression. When matched OR and *P* values were calculated, data for matched control patients were excluded for patients with missing exposure data, and control subjects with missing data were excluded from analyses; however, matched patients or other control subjects with available data were included.

[b] Travel outside home township (for rural patients) or outside home city (for urban patients) for >24 h during the 2 weeks prior to the patient's illness onset.

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

[d] Includes ducks and geese.

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

[f] A family cluster was reported in Yu et al. [15].

[g] A family cluster consisting of confirmed son and his father was reported in Wang et al. [8].

significant risk factor for influenza H5N1, consistent with previous studies [13, 14]. Close indirect exposure to sick and/or dead poultry was also reported in a descriptive study of Indonesian influenza H5N1 [9]. This could reflect inhalation of aerosolized material contaminated with influenza 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]; however, our study design did not address these mechanisms.

Our finding that visiting a wet poultry market during the 2 weeks before illness onset was a significant risk factor for influenza H5N1 is consistent with findings from a case-control study conducted during the outbreak of influenza H5N1 in Hong Kong in 1997 [12]. Although widespread poultry deaths from influenza H5N1 were noted in wet markets during the outbreak in Hong Kong, this has rarely been observed in urban China. Wet poultry markets are considered to be a reservoir and amplifier of avian influenza A viruses, because avian host species are present together in a high-density setting that can facilitate viral persistence, cross-species infection, and genetic reassortment [23, 24]. Influenza H5N1 viral RNA was detected in an environmental specimen collected from a goose cage at a market that an urban patient with influenza H5N1 had visited before illness onset [25], which suggests that influenza H5N1 virus transmission through environmental contamination may occur in urban areas of China.

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

Chinese patients with influenza H5N1 comprised 2 distinct populations with respect to poultry exposures. Most rural Chinese persons 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 on where the patients lived. Most urban patients had not been 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 been exposed to backyard poultry and to sick or dead poultry. This suggests that public education and interventions to control disease should target different settings. Rural patients were less educated, poorer, and more likely to lack an indoor water supply, compared with urban patients—similar to risk factors identified in Vietnam [14]. Because of the exposure differences between rural and urban patients, we performed analyses stratified by patient 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 a greater risk to public health, compared with contact with chickens. Studies from Vietnam, Thailand, and southern China have documented that domestic ducks and geese can be infected with highly pathogenic avian influenza H5N1 viruses without apparent symptoms [29–31]. Earlier studies, conducted during 1997–2004, suggested that most influenza H5N1 viral shedding by domestic ducks was in feces, but more recently, a great amount of influenza 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 influenza 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 influenza H5N1 disease, but raising only backyard chickens was not a risk factor. This finding suggests that domestically raised waterfowl exposure may pose a greater risk of avian-to-human transmission, compared with exposure to backyard chickens in rural areas.

In China, a national influenza H5 poultry vaccination program was implemented in 2005 [33]; after that, subsequently documented decreases in outbreaks of influenza H5 among poultry were noted [1]. However, the effectiveness of poultry H5 vaccination to reduce the risk of influenza 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 influenza H5 vaccination of poultry, 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 virus for transmission to humans was suggested by recent field evidence [37–39]. However, cases of influenza H5N1 continued to occur in China during 2006–2008, despite the national poultry H5 vaccination program. A simulation study revealed

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

Although human influenza H5N1 disease is very rare and persons with the risk factors that we identified seldom develop influenza H5N1 virus infection [41], interventions based on our findings may help prevent further influenza H5N1 virus transmission to humans in China. Ongoing 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 using 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 Special Administrative Region, such as only selling H5-vaccinated poultry, segregating bird species, improving biosecurity, and having central poultry slaughtering locations, regular disinfection, and a monthly rest day [42–44]. In addition, the feasibility of the wearing of protective masks or respirators by workers and visitors to wet poultry markets could be considered.

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