CONCISE COMMUNICATIONS

Case-Control Study of Risk Factors for Avian Influenza A (H5N1) Disease, Hong Kong, 1997

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In May 1997, a 3-year-old boy in Hong Kong died of a respiratory illness related to influenza A (H5N1) virus infection, the first known human case of disease from this virus. An additional 17 cases followed in November and December. A case-control study of 15 of these patients hospitalized for influenza A (H5N1) disease was conducted using controls matched by age, sex, and neighborhood to determine risk factors for disease. Exposure to live poultry (by visiting either a retail poultry stall or a market selling live poultry) in the week before illness began was significantly associated with H5N1 disease (64% of cases vs. 29% of controls, odds ratio, 4.5, $P = .045$). By contrast, travel, eating or preparing poultry products, recent exposure to persons with respiratory illness, including persons with known influenza A (H5N1) infection, were not associated with H5N1 disease.

During the twentieth century, novel influenza viruses have led to 3 global influenza epidemics, known as pandemics. In 1918–1919, the appearance of influenza A (H1N1) virus (i.e., "Spanish flu") led to >20 million deaths worldwide and >500,000 deaths in the United States [1]. In 1957 and 1968, pandemics related to the appearance of influenza A (H2N2) and influenza A (H3N2) viruses, respectively, together resulted in >100,000 deaths in the United States alone [1, 2]. Each pandemic resulted in significant social disruption and economic losses and in increased morbidity and mortality.

Because of these precedents, an outbreak of 18 cases of respiratory disease in Hong Kong caused by influenza A (H5N1) viruses, which previously were known only to infect avian species and cause disease among domestic birds, raised tremendous concerns about the potential for another pandemic [3–7]. We conducted this study in January 1998 to determine risk factors for persons who had become ill from influenza A (H5N1) infections.

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Informed consent was obtained from study participants or their legal guardians in accordance with US Department of Health and Human Services guidelines.

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Methods

Patient sample. We studied 15 subjects who had been hospitalized for febrile respiratory illness in Hong Kong during November ($n = 4$) and December ($n = 11$) 1997 and who had either viral culture or serologically confirmed (4-fold rise in antibody titer) influenza A (H5N1) infections. The median age of the subjects was 6 years (range, 1.5–60), 8 (53%) were <7 years old, and 9 (60%) were female. They lived throughout Hong Kong, with no obvious geographic clustering.

Three people with culture-confirmed H5N1 illness were excluded from this study. A 3-year-old boy (the index patient) was excluded because 8 months had elapsed since his death. A 54-year-old man was excluded because a proxy could not provide sufficient information for the study, and a 34-year-old woman was excluded because no proxy could be found. All 3 of these cases were fatal.

Case definition. A case of H5N1 illness was defined as fever plus cough or sore throat and either a positive viral culture for influenza A (H5N1) virus or a 4-fold rise in H5-specific antibody titer [8].

Selection of controls. We recruited ≥2 controls for each case subject. To recruit controls, the study team first identified the apartment buildings surrounding each of the case subjects' residences and then randomly selected one of the buildings (by drawing numbers). Next, a floor within the building was randomly selected, and the study team went from door-to-door asking for volunteer controls.

Two controls were matched by sex and age (within 1.5 years for case subjects <18 years old and within 5 years for adults ≥18 years old) to each case subject. In addition, for 2 case subjects who were housekeepers, we selected 2 additional housekeepers as controls, to increase the study's ability to identify home and food handling

activities as risk factors. This resulted in a total of 4 controls for each housekeeper and 2 controls for each of the other case subjects.

Interviews. A standardized questionnaire developed by the field team was used to collect information about demographic characteristics, daily activities, travel, shopping habits, visits to places where live poultry were kept (e.g., poultry stalls, retail markets, wholesale markets or poultry farms) in the week before illness, eating habits, food preparation activities related to poultry, exposure to other animals, and exposure to other humans with respiratory illnesses. The questionnaire was developed in English and was administered by public health workers to participants in the language appropriate for the interview. All the interviews were conducted in either Cantonese or English, except for 1 conducted in Vietnamese.

We asked an adult household member who was closely familiar with the case subject to be interviewed as a proxy, for any case patient who had died, was incapacitated, or was <12 years old. We also used an adult proxy to interview controls who were younger than 12 years. For questions in which case subjects were asked about activities and exposures occurring in "the week before your illness," controls were asked about the same activities and exposures "during an average week" of the same month in which their matched case subject became ill.

Specimen collection and testing. The infection status of each case subject was established in earlier investigations either by viral culture or by serologic tests showing a 4-fold rise in H5-specific antibody [8]. The viral cultures were conducted at the Hong Kong Department of Health Government Virology Laboratory, Hong Kong, and the serologic tests were conducted at the Influenza Branch, Centers for Disease Control and Prevention (CDC), Atlanta. Controls were asked to donate 1 blood sample to determine whether they had antibodies to H5N1. The serum specimens were tested at the CDC by a microneutralization assay that detected influenza A (H5N1)–specific antibody. Positive sera were retested by a Western blot assay. A positive H5N1 antibody test required a microneutralization antibody titer $\geq$80 on 2 occasions and a positive Western blot [8].

Data analysis. Common odds ratios (ORs) and confidence intervals (CIs) were tested by an exact test, in a matched case-control design using the algorithm developed by C. R. Mehta, N. R. Patel, and R. Gray [9]. Differences in age were tested by the Wilcoxon rank sum test. In matched analyses, a variable was not analyzed if a case was missing data for that variable. However, if 1 of the matched controls was missing data for a variable, then only that matched control was dropped from the analysis.

Results

None of the controls had antibody to influenza A (H5N1) virus. Thirteen of 15 case subjects had influenza A (H5N1) infections confirmed by culture, and 2 had infections confirmed by a 4-fold rise in H5-specific antibody. Case subjects (12/15) were interviewed by proxy significantly more often ($P = .003$) than were controls (21/41). Otherwise, case subjects and controls were similar by income, type of residence, number of persons per square meter of home living space, chronic illness, and smoking (table 1).

In the matched-pair analysis, 9 (64%) of 14 case patients and 11 (29%) of 38 controls reported poultry exposure (visiting either a poultry stall or a retail market selling live poultry) in the week before illness onset (OR, 4.5; 95% CI, 1.2–21.7; $P = .045$; table 2). None of the case subjects reported visiting a poultry farm or wholesale market in the week before illness, and none of the controls reported visiting a poultry farm or wholesale market during an average week of the same month in which their matched case subject became ill.

Table 1. Baseline characteristics of influenza A (H5N1) case-control study subjects.

Baseline characteristic	No. (%) Case patients ($n = 15$)	No. (%) Controls ($n = 41$)	P[a]
Age, years			
1–10	8 (53)	22 (54)	
11–20	3 (20)	7 (17)	
20–60	4 (27)	12 (29)	.860
Sex (male)	6/15 (40)	16/41 (39)	.948
Any chronic medical condition	0/15	0/41	NA
Smoking	0/15	3/41 (7)	.161
Monthly income (<HK $20,000)	5/14 (36)	18/35 (51)	.443
Housing (private apt. vs. other)[b]	5/15 (33)	20/41 (49)	.282
Rented home	6/14 (43)	21/37 (57)	.311
Individuals/m^2 (<1 person/9.29 m^2)	9/15 (60)	25/41 (61)	.999

[a] Exact method except for ages, which were compared by Wilcoxon rank sum test. NA, not available.
[b] Apt., apartment.

Activities related to poultry preparation or eating, contact with wild birds, travel, or exposure to a person with an influenza-like illness were not significantly different between case subjects and controls. One (8%) of 13 case subjects and none of 35 controls reported exposure to a person known to have been infected with influenza A (H5N1) virus ($P = .5$). Playing in an indoor playground the week before illness was reported more frequently by controls (32%) than by case subjects (0%) (OR, 0.0; 95% CI, 0.0−0.5; $P = .013$).

Discussion

In January 1998, we conducted this case-control study to identify risk factors among persons hospitalized for H5N1 disease during the first reported human outbreak of influenza caused by an avian influenza A virus. Influenza A (H5N1) disease was associated with recent exposure to live poultry but not to several other plausible risk factors or exposures, including activities related to the eating or home preparation of fresh poultry, exposure to other birds, travel (including to China), or recent contact with persons with respiratory illness. Although 1 case patient had contact with another child with influenza A (H5N1), raising the possibility of human-to-human transmission, both children also lived near a poultry stall from which H5N1 virus was cultured. We also found a negative association between case subjects and playing in an indoor playground that was unexpected and difficult to explain. We had hypothesized that activities in enclosed spaces with large groups of people might predispose toward infection. The association

Table 2. Activities and exposures associated with influenza A (H5N1) disease.

Activity and exposure	No. (%) with reported exposure			
	Case patients (n = 15)	Controls (n = 41)	OR (95% CI)	P[a]
Exposure to poultry				
Exposed to live poultry in market[b]	9/14 (64)	11/38 (29)	4.5 (1.2–21.7)	.045
Consumed poultry in restaurant	6/12 (50)	9/31 (29)	2.9 (0.6–14.9)	.375
Consumed poultry organs or poultry	1/14 (7)	4/38 (11)	0.6 (0.0–7.5)	.999
Consumed undercooked poultry products	5/11 (45)	10/29 (34)	1.9 (0.4–11.2)	.707
Household member cooked poultry products	2/13 (15)	11/33 (33)	0.2 (0.0–1.3)	.193
Household member in poultry industry	0/14	0/38	Undefined	NA
Exposure to human illness				
Anyone in flat had influenza-like illness[c]	5/15 (33)	15/40 (38)	0.8 (0.2–2.8)	.999
Contact with known H5N1 case	1/13 (8)	0/35	+Inf (0.1 to +Inf)[d]	.500
Other exposure				
Travel outside Hong Kong	0/14	3/37 (8)	0.0 (0.0–4.9)	.800
Selected outdoor activities[e]	2/14 (14)	8/37 (22)	0.0 (0.0–2.5)	.225
Live birds in home	6/12 (50)	14/31 (45)	1.4 (0.3–6.4)	.901
Other animals in home	2/13 (15)	1/33 (3)	2.5 (0.1–97.4)	.999
Played in indoor playground	0/15	13/41 (32)	0.0 (0.0–0.5)	.013
Household uses soap to clean[f]	5/14 (36)	25/38 (66)	0.3 (0.1–1.0)	.058

NOTE. OR, odds ratio; CI, confidence interval; NA, not available.

[a] Exact method.

[b] Includes visiting poultry stall, retail or wholesale market selling live poultry, or poultry farm in week before illness.

[c] Influenza-like illness includes anyone with fever and cough or sore throat.

[d] +Inf, an unknown upper bound (positive infinity) for the CI.

[e] Includes activities such as visiting aviary, feeding wild birds in park, and/or having picnic in park.

[f] Uses soap and water to clean knife after preparation of poultry.

may be related in some way to the socioeconomic status of the study participants, but our attempts to stratify the analysis by socioeconomic status were hampered by the small number of case subjects.

At the time of this study, indirect evidence pointed to an association between infections in chickens and humans; however, no epidemiologic evidence had been established that directly linked the two. Three outbreaks of influenza A (H5N1) disease had occurred among chicken flocks on 3 poultry farms in the New Territories of Hong Kong during March–May 1997 and preceded illness in the index human case in May 1997 [10]. Investigations at that time could not establish a direct link between the index case and exposure to the infected poultry. From June–October 1997, no new poultry outbreaks or infections were identified, despite active serosurveys of local poultry farms (L. Sims, Hong Kong Department of Agriculture and Fisheries, personal communication), and no new human cases were detected. However, in November and December, new human H5N1 cases were detected during the same time period when influenza A (H5N1) viruses were cultured from both live chickens and chicken feces obtained from retail poultry stalls and wholesale poultry markets in Hong Kong. Moreover, preliminary genetic sequence data available at the time or subsequently for 16 viruses isolated from the human cases indicated that all were avian-like viruses, without evidence of genetic reassortment with circulating human influenza A viruses or cumulative genetic changes suggestive of adaptation to humans [4, 11, 12] (K. Subbarao, CDC, personal communication).

Avian influenza viruses have rarely been reported to cause illness in humans. Two cases of conjunctivitis were reported in association with avian influenza A (H7N7) viruses [13, 14]. In 1992, a report indicated that antibody to several avian influenza virus subtypes was found in a serosurvey of rural residents in southern China [15]; however, no associated disease was reported, and the high rates of antibody to several different influenza A virus subtypes have not been independently confirmed.

Our study had several potential limitations. The number of case subjects was small, limiting the power of the study to demonstrate significant associations. Case-subject interviews were conducted more frequently by proxy than were control interviews. However, this was unavoidable given the deaths among case subjects. Although the potential effect of the differential use of proxies in our study is unknown, we believe that proxies were likely to be less aware of specific exposures and activities, resulting in a bias toward finding no association between an exposure and illness. In addition, Hong Kong media reports openly speculated on the possibility that poultry markets were a source of the epidemic. Although this may have led to differences in recall between case subjects and controls, the media also speculated that activities such as eating fresh poultry or eating poultry in restaurants were sources of infection, but these exposures were not significantly associated with disease. Finally, interviews were conducted in Cantonese, English, and (in 1 case) Vietnamese. Although the interviews were done in 3 different languages, potentially leading to variations in phrasing, all the interviewers read the questions off a standardized questionnaire and were fluent multilingually and experienced in conducting health interviews.

Although the results indicated that exposure to live poultry was a major risk factor for influenza A (H5N1) infection, the exact mode of virus transmission remains uncertain. Human

influenza viruses normally are transmitted by aerosolized droplets. However, birds excrete influenza viruses in feces, and it is unknown whether the human infections occurred by inhalation of aerosolized virus, by direct contact with virus in poultry feces, or by some other route. Poultry-to-human transmission of this virus is unlikely to lead to pandemic influenza, but avian influenza viruses have the potential to either reassort with human influenza strains or to mutate and become more transmissible among humans. This outbreak and these considerations strongly emphasize the need for continued high levels of awareness and surveillance to monitor for emerging influenza viruses.

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