Antibody Response in Individuals Infected with Avian Influenza A (H5N1) Viruses and Detection of Anti-H5 Antibody among Household and Social Contacts

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The first documented outbreak of human respiratory disease caused by avian influenza A (H5N1) viruses occurred in Hong Kong in 1997. The kinetics of the antibody response to the avian virus in H5N1-infected persons was similar to that of a primary response to human influenza A viruses; serum neutralizing antibody was detected, in general, ⩾14 days after symptom onset. Cohort studies were conducted to assess the risk of human-to-human transmission of the virus. By use of a combination of serologic assays, 6 of 51 household contacts, 1 of 26 tour group members, and none of 47 coworkers exposed to H5N1-infected persons were positive for H5 antibody. One H5 antibody–positive household contact, with no history of poultry exposure, provided evidence that human-to-human transmission of the avian virus may have occurred through close physical contact with H5N1-infected patients. In contrast, social exposure to case patients was not associated with H5N1 infection.

The first known case of human infection with avian influenza A (H5N1) virus occurred in a 3-year-old male resident of Hong Kong who died in May 1997 of acute respiratory distress syndrome and complications resulting from Reye's syndrome [1–3]. In the 2 months preceding the child's fatal illness, H5N1 viruses were isolated from poultry farms in the New Territories, Hong Kong. In November and December of 1997, 17 additional human cases of H5N1 infection occurred in Hong Kong. H5N1 viruses were isolated from birds at wholesale and retail markets in Hong Kong during this period [4]. Of a total of 18 human cases, 6 were fatal.

Avian influenza A viruses, including those that are highly pathogenic in poultry, have not previously been associated with respiratory disease in humans, although H7 viruses have been associated with 2 unrelated cases of human conjunctivitis [5, 6]. Studies performed on individuals involved in the depopulation of chickens infected with a highly pathogenic H5N2 virus in the northeast United States in 1983–1984 found no evidence of human infection with this avian virus [7]. Experimental infection of human volunteers with high doses of avian influenza viruses of the H4N8, H6N1, and H10N7 subtypes resulted in limited virus replication, minimal clinical symptoms, and no detectable hemagglutination-inhibition (HI) antibody response [8]. In contrast, 18 of the individuals who were infected with H5N1 virus in Hong Kong were hospitalized with mild to fatal respiratory illness.

We sought to characterize the primary serologic antibody response to the avian H5N1 virus in these infected individuals and to investigate the possibility of human-to-human transmission of the virus to assess its pandemic potential. An investigation of seroprevalence of anti-H5 antibody among contacts of the first H5N1 virus–infected case patient, individuals in contact with poultry, and controls suggested that the prevalence of antibody to H5N1 virus was higher among individuals exposed to infected poultry than in individuals exposed to the infected child [9]. However, the possibility of human-to-human transmission of the virus could not be excluded. When 17 additional H5N1 cases were identified 6 months after the first case, we conducted this study to determine the extent of human-to-human transmission of the virus to contacts of the H5N1-infected patients. We report here the serum antibody responses of individuals confirmed to be infected with influenza A H5N1 virus and the seroprevalence of H5N1 antibody in household and social contacts exposed to persons ill with influenza A (H5N1) disease.

Received 5 April 1999; revised 30 July 1999; electronically published 12 November 1999.

Presented in part: American Society for Virology annual meeting, Vancouver, BC, Canada, July 1998.

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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The Journal of Infectious Diseases 1999; 180:1763–70
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0022-1899/1999/18006-0002$02.00

Materials and Methods

Investigation of Case Patients

A case of H5N1 disease was defined as fever (temperature ⩾38°C) with cough, sore throat, or both of these symptoms and laboratory confirmation by a positive culture for influenza A H5N1 virus at the Government Virus Unit in Hong Kong (table 1) or a ⩾4-fold rise in titer to H5N1 virus by a microneutralization assay [10]. Staff from the Hong Kong Department of Health interviewed each person who was ill from H5N1 virus or an adult member of the household if the case patient was <12 years of age or had died. Information was collected on daily activities, exposure to birds and poultry, and exposure to humans with respiratory illness. An attempt was made to collect serum samples as early as possible from individuals who were suspected to be infected with H5N1 virus. The onset of symptoms was established from the clinical history of the case patient at the time of admission to a hospital. Sera collected ⩽7 days after symptom onset were considered acute-phase (S1) samples. An attempt was made to collect a convalescent-phase (S2) serum sample from each case patient ⩾14 days after symptom onset. For some case patients who died of their illness, collection of S2 sera ⩾14 days after symptom onset was not possible. Paired sera were obtained from 8 individuals. Because of delays in the diagnosis of this novel virus infection, single serum samples only were collected from 6 individuals ⩾11 days after symptom onset. For 2 other case patients, whose conditions deteriorated rapidly, only S1 samples were obtained.

Cohort Studies

Household and nonhousehold contacts. Information on daily activities, history of recent respiratory illness, and exposure to birds and poultry was collected from household and nonhousehold contacts of H5N1-infected persons during interviews conducted by staff from the Hong Kong Department of Health. Household contacts were defined as individuals who lived with an H5N1-infected person for part or all of the case patient's infectious period. The infectious period was defined as 1 day prior to illness onset through day 14 of illness. The timing and number of sera collected from contacts varied and depended on contact compliance and availability. Two or 3 sequential serum samples were collected from each of 42 contacts. Most paired sera were collected ⩽11 days and ⩾21 days after initial exposure to a case patient. Four contacts of case patient 6 had sera collected 26 and 51 days after their initial exposure. Single sera from 12 contacts were collected ⩾13 days after exposure; single sera from 4 contacts of case patient 7 were collected 8 days after exposure.

Tour group cohort. A tour group cohort was exposed to case patient 4, who became ill with fever on November 24 and who took a 4-day organized tour to an Asian country beginning on November 26. Paired sera were collected 12–15 days and 35–39 days after first exposure to the case patient. Information was collected on age, sex, smoking habits, occupation, travel history, medical history, history of recent respiratory illness, exposure to poultry and other birds, and extent and type of exposure to the case patient (i.e., eating at the same table, talking with the ill person, or being in close proximity when the case patient coughed).

Bank coworker cohort. Case patient 6 became ill from H5N1 infection on November 17 and continued to work at his office for 5 days while ill. Completed questionnaires were collected from 47 office coworkers. A single serum sample was collected from each office worker 36 days after exposure to the case patient. Individuals were considered to have been exposed to the case patient if, while at work between 17 and 21 November 1997, they worked in the same room, had lunch, or spoke with the case patient or were in close proximity when the case patient coughed.

Definition of poultry exposure. Household contacts were asked about their exposure to birds and poultry in the weeks preceding

Table 1. Characteristics of 17 patients infected with avian influenza A (H5N1) virus.

Case patient	Age (y), sex	No. of days in hospital	Outcome[a]	Exposure to poultry	No. of household contacts[b]
2	2, M	2	Recovered	No def. hist.	4
3	13, F	25	MV; died	No def. hist.	3
4	54, M	6	MV; died	No def. hist.	1 (2)
5	5, F	19	Recovered	Yes	6 (3)
6	37, M	15	Recovered	Yes	4
7	24, F	116	MV; recovered	No def. hist.	4
8	2, M	16	Recovered	Yes	—[c]
9	4, M	18	Recovered	No def. hist.	3 (2)
10	1, M	2	Recovered	No def. hist.	2
11	3, F	2	Recovered	No def. hist.	3
12	60, F	5	MV; died	No def. hist.	5
13	25, F	24	MV; died	Yes	2 (2)
14	14, F	18	Recovered	Yes	4
15	3, M	9	Recovered	No def. hist.	4
16	19, F	82[d]	MV; recovered	Yes	4
17	6, F	11	Recovered	Yes	2
18	34, F	14	MV; died	No def. hist.	0

NOTE. Case patient 1 has been reported elsewhere [9]; def. hist, definite history.

[a] MV, the patient required mechanical ventilation.

[b] Value in parentheses indicates number of nonhousehold contacts.

[c] The household and nonhousehold contacts of case patient 8 are the same as those of case patient 5.

[d] Patient was readmitted to hospital for periods of 20 and 32 days after development of a pneumothorax on 2 occasions.

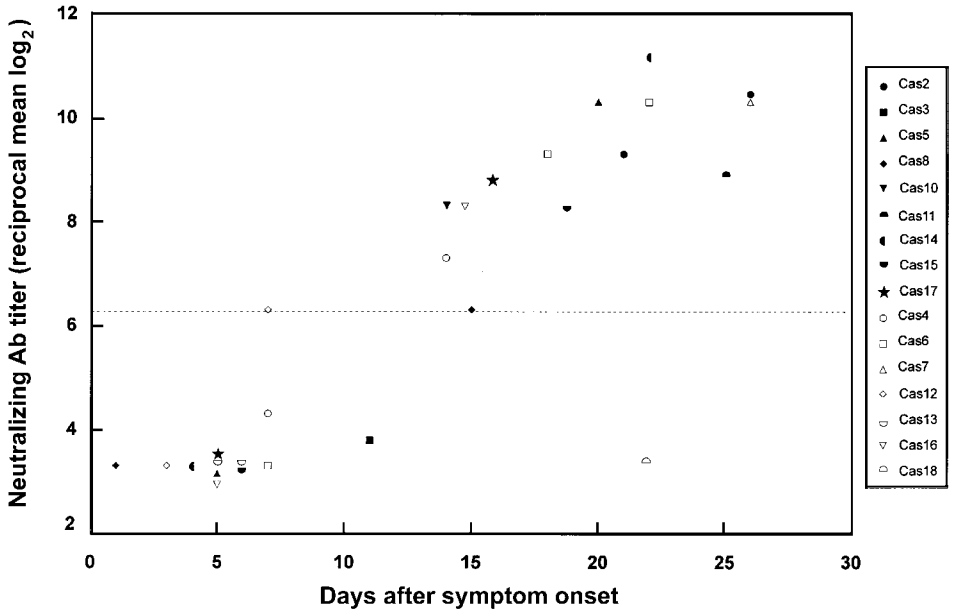

Figure 1. Kinetics of serum neutralizing antibody response to infection with avian influenza A (H5N1) virus. Serum samples from 16 H5N1 case patients were tested in a microneutralization assay by use of the A/Hong Kong/156/97 virus. Values represent the $\log_2$ mean titer of duplicate assays. Closed symbols represent case patients ⩽14 years of age; open symbols represent case patients >14 years of age. The dotted line denotes a titer of $\log_2 6.3 = 80$. Sera with titers ⩾80 were considered positive for H5-specific antibody [9]. Serum samples were not collected from case patient 9.

the H5N1 illness in their household. Persons in both the tour group and coworker cohorts were asked about having ever lived or worked on a poultry farm and whether, since November 1, 1997, they had shopped at a market or stall (an urban retail business) that sold live poultry, had live or freshly butchered poultry in the home, had poultry butchered at the home, or had contact with any poultry or pet birds that appeared sick, had yellow diarrhea (a characteristic symptom of birds infected with highly pathogenic H5 virus), or had died.

Serologic Analysis

A microneutralization assay or an H5-specific ELISA, each followed by confirmation with a Western blot assay, was used to detect antibody responses in this study. The sensitivity and specificity of these assays for the detection of anti-H5 hemagglutinin (HA) antibody in adults and children (⩽14 years of age) have been described elsewhere [10]. Sera were tested in parallel by use of the H5N1 prototype virus A/Hong Kong/156/97 as the test virus, in biosafety level 3+ laboratories at the Centers for Disease Control and Prevention (CDC; Atlanta, GA), and the nonpathogenic avian H5N3 virus A/Duck/Singapore-Q/F119-3/97, at the Government Virus Unit, Hong Kong Department of Health. Sera were considered to be positive in the microneutralization assay if anti-H5 titers of ⩾80 were obtained in 2 independent microneutralization assays performed at 1 or both testing facilities. A confirmatory Western blot assay, performed at the CDC on all sera that were positive by the microneutralization assay, used a highly purified baculovirus-expressed HA protein from A/Hong Kong/156/97 virus (provided by Dr. Bethanie Wilkinson, Protein Sciences, Inc, Meridan, CT) to detect antibody in sera diluted 1/100. Sera from adults had to test positive by both microneutralization assay and Western blot to be considered positive for anti-H5 antibody. Adults ⩾60 years of age were excluded from the analyses because of decreased specificity of the microneutralization and Western blot assays for this age group [10]. The neutralizing antibody titers of the case patients (figure 1) are expressed as the mean of 2 determinations.

Sera from persons ⩽14 years old were also tested by an indirect ELISA by use of purified baculovirus-expressed H5 HA (1 μg/mL) as the coating antigen and horseradish peroxidase–conjugated goat anti–human IgG or IgM as the detecting antibody. The ELISA is more sensitive than the microneutralization assay for detecting H5 HA-specific IgG or IgM in this age group [10] and was used to test specimens from case-patient contacts ⩽14 years of age. The Western blot (IgG- or IgM-specific) was used to confirm a positive ELISA titer (⩾1600) in these sera. Sera that were positive for IgG or IgM in both ELISA and Western blot were considered positive for anti-H5 antibody.

Statistical Analysis

Two by two contingency tables were tested by Fisher's exact test. The age distribution between groups was tested by use of the Wilcoxon rank sum test. $P \leqslant .05$ was considered statistically significant.

Results

Description of 17 H5N1 Case Patients

A summary of the 17 H5N1-infected patients is shown in table 1. Fifteen of the case patients were confirmed by culture isolation of influenza A (H5N1) virus. Case patients 6 and 17 were confirmed by seroconversion for antibody to H5N1. Case patients 5 and 8 were cousins who lived in the same household during the time that case patient 5 was symptomatic. Other than these 2 cases patients, there were no clusters or associations among the case patients. In contrast to the previously reported fatal index case patient, a 3-year-old boy [9], the 8 children in this study <12 years of age all survived the H5N1 infection and made complete recoveries. However, infection was more severe, requiring mechanical ventilation, in 7 of 9 individuals >12 years of age. Five of these case patients died, whereas the remaining 2 case patients were hospitalized for extended periods but eventually recovered. Clinical details of 11 H5N1 case patients have been described elsewhere [11].

Serologic Response to H5N1 Virus Infection

The kinetics of the induction of neutralizing antibody to H5N1 virus in 16 juvenile and adult patients is shown in figure 1. In general, a neutralizing antibody titer of ≥ 80 ($\log_2 \geq 6.3$) was detected in sera collected ≥ 14 days after the onset of clinical symptoms, with neutralizing antibody titers of 80–2560. Titers ≥ 640 were observed in both children and adults ≥ 20 days after onset of symptoms. Several exceptions were noted. Case patient 2, a 60-year-old woman, seroconverted 7 days after onset of respiratory symptoms. In contrast, case patient 18, a 34-year-old woman with systemic lupus erythematosus, had no detectable neutralizing antibody 23 days after onset of symptoms, perhaps because of her underlying illness or treatment with steroids. The single serum specimen collected 11 days after symptom onset from case patient 3 was also negative for neutralizing antibody to A/Hong Kong/156/97 virus.

The H5-specific ELISA IgG and IgM antibody responses of 8 children are shown in figure 2. All 8 children showed IgG antibody titers of $\geq 25{,}600$ at 11–14 days after onset of symptoms. H5-specific IgM was detected in 7 of the 8 children. Both H5-specific IgG and IgM were detected in the neutralizing antibody-negative serum sample from case patient 3, collected 11 days after symptom onset. Four of 5 adults tested were positive for H5-specific IgG, and 3 of 5 adults were positive for IgM (data not shown). The serum from case patient 18 that tested negative for H5N1-neutralizing antibody was also negative for H5-specific IgG and IgM. All sera that tested positive for IgG or IgM by ELISA also reacted with the purified H5 HA, by Western blot (data not shown).

Prevalence of Antibody to H5N1 Virus among Individuals Exposed to Case Patients

Three cohorts of individuals who had been exposed to the H5N1 case patients were studied to evaluate the risk of human-to-human transmission of influenza A (H5N1) viruses. The groups included household and nonhousehold contacts exposed to an H5N1 case patient, members of a tour group exposed to case patient 4, and office workers exposed to case patient 6. The characteristics of the 3 cohorts are presented in table 2.

Household and nonhousehold contacts. A total of 51 household contacts and 9 nonhousehold contacts of 16 case patients were evaluated (table 1). Household contacts consisted mainly of family members (table 2). Household contacts of case patients 2 and 15 included a nonfamily boarder and a domestic helper, respectively. Case patients 7 and 13 were employed as domestic helpers, and their household contacts were their respective employers and their families. Nonhousehold contacts included adult children who visited a case patient in the hospital, household visitors, or a friend who had social contact with a case patient (table 1). The nonhousehold contacts were 6–50 years of age, with a median age of 28 years; 44% were male. None of the 9 nonhousehold contacts analyzed were positive for anti-H5 antibody.

Six (12%) of 51 household contacts were positive for antibody to H5N1 virus. The characteristics of the antibody-positive individuals are shown in table 3. None of the adult contacts who were seropositive for H5 antibodies were symptomatic during the weeks after exposure to the case patients. One of the seropositive children was case patient 8, a 2-year-old male household contact of case patient 5, a 5-year-old girl. For 2 days after the onset of symptoms, including fever, cough, sore throat, and vomiting, case patient 5 stayed at the home of case patient 8 and another cousin, a 3-year-old girl. Case patient 5 was admitted to a hospital on the evening of the second day of her illness. Case patient 8 became symptomatic 3 days after his initial exposure to case patient 5 and was admitted to a hospital. Influenza A (H5N1) virus was isolated from a nasopharyngeal aspirate obtained from case patient 8, and seroconversion was documented with sera collected 16 days after exposure to case patient 5. The 3-year-old female cousin developed symptoms 1 day after case patient 8 and was also admitted to a hospital. In contrast to case patients 5 and 8, this child remained serologically negative for H5 antibody

Two other children, daughters of case patient 6, were also found to have antibodies to H5N1 virus, although neither child was reported to have a recent respiratory illness. Throat swab specimens collected concurrent with the first serum specimen were culture negative. One child was positive for H5-specific antibody in both serum samples, whereas the other child seroconverted between the first and second serum collection.

Tour group members. Paired serum samples and completed questionnaires were collected from 26 individuals who took part in a 4-day tour with case patient 4. All tour members rode

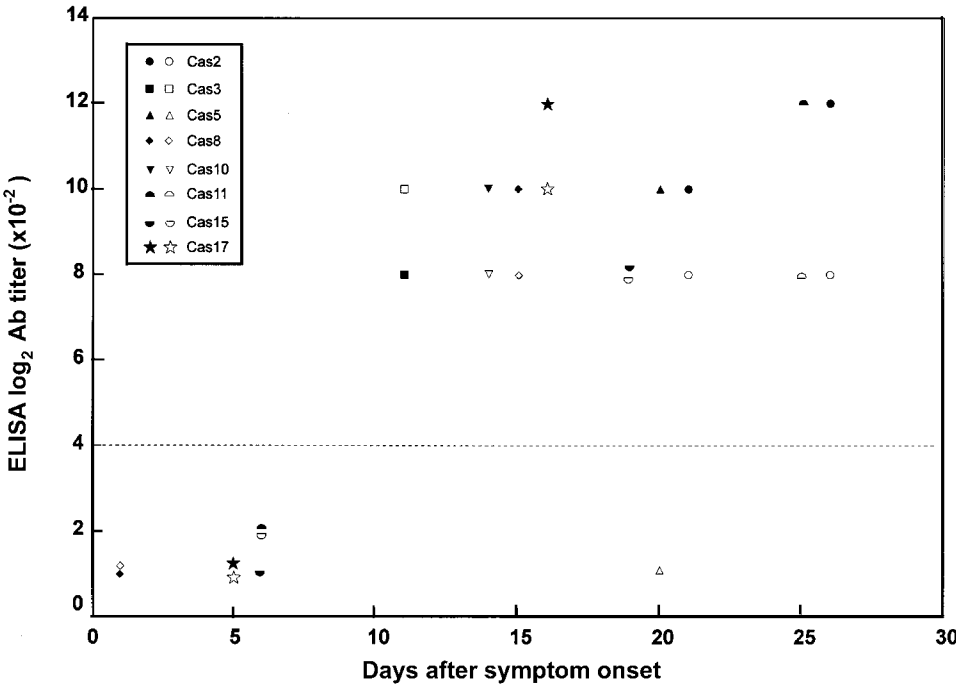

Figure 2. Kinetics of H5-specific ELISA antibody response in children infected with avian influenza A (H5N1) virus. Closed symbols represent anti-H5 IgG antibody; open symbols represent anti-H5 IgM antibody. The dotted line denotes a titer of $(\log_2 4.0) \times 10^{-2} = 1600$. Children's sera with ELISA titers $\geq$1600 were considered positive for H5-specific antibody [9]. Serum samples from case patient 14 could not be tested because of insufficient volumes.

buses daily with case patient 4 (the longest ride was ~3 h) and shared 1 or 2 airline flights of 3.5 h duration. Twenty-two tour members either shared a meal, talked with the H5N1-infected person, or were close by when the ill person coughed. One (4%) of 26 individuals was positive for neutralizing antibody to H5N1 virus in the first serum sample, collected 12 days after initial exposure to the case patient. This serum sample was also positive by Western blot. However, a second sample, collected 23 days later, was positive by Western blot but not by microneutralization assay and therefore was considered to be anti-H5–antibody negative. Because the first serum fulfilled the criteria for positivity, the individual was considered to be positive for antibody to H5N1 virus. The H5-antibody–positive individual had been exposed to the case patient on the airplane, on the tour buses, by eating a meal and talking with him, and by being nearby, but at a distance of >2 m, when the case patient coughed. However, 28% of the H5-antibody–negative individuals were also exposed to the case patient by all of the latter 3 criteria. The H5-antibody–positive individual did not report a respiratory illness that began after exposure to case patient 4 and had been exposed to poultry in the 2 months prior to the collection of serum samples.

Bank coworkers. The characteristics of 23 workers exposed to case patient 6 were similar to those of 24 nonexposed workers, except that the case-patient–exposed workers were also more likely to have been exposed to poultry (table 2; $P = .05$). None of the 24 nonexposed or the 23 exposed individuals had detectable anti-H5N1 neutralizing antibodies.

Prevalence of Anti-H5 Antibody in Cohort Participants, Stratified by Poultry Exposure

Because exposure to poultry was shown to be a risk factor for infection with influenza H5N1 viruses in a case-control study [12], the results were also stratified by poultry exposure. (table 4). Twenty-one percent of household contacts exposed to poultry were positive for anti-H5 antibody, compared with only 5% of those not exposed to poultry ($P = .13$). Although these results were not statistically significant, because of the small numbers of individuals in the cohort, the trend was toward a higher incidence of H5 antibody among individuals exposed to poultry. The only H5-antibody–positive individual in the tour group cohort was also exposed to poultry.

Discussion

The emergence of influenza A (H5N1) virus in humans in Hong Kong in 1997 provided a unique opportunity to assess the primary immune response to respiratory infection with an avian influenza virus. By examining the serologic responses of

Table 2. Characteristics of the household contacts, tour group, and coworker cohorts.

Description	Household contacts (case patients 2–17) (n = 51)	Tour group cohort (case patient 4) (n = 26)	Coworker cohort (case patient 6)	
			Exposed (n = 23)	Not exposed (n = 24)
Median age, years (range)	32 (0.6–58)	32 (22–55)	36 (22–58)	40 (25–58)
No. (%) male	23 (45)	9 (35)	13 (57)	16 (67)
No. (%) with chronic illness	ND	3 (12)	7 (30)	8 (33)
No. (%) of smokers	ND	2 (8)	1 (4)	6 (25)
No. (%) with poultry exposure	24 (52)[a]	16 (62)	9 (39)[b]	3 (13)

NOTE. ND, not determined.

[a] Status of exposure to poultry was determined for 46 household contacts.

[b] Difference in poultry exposure between groups exposed or not exposed to case patient was significant (P = .05).

16 H5N1-infected patients, we established a profile of the kinetics of the primary neutralizing antibody response to the avian virus. Interestingly, the kinetics of the antibody response to avian H5N1 virus was similar to that described for a primary response to a human influenza A virus [13]. In general, the neutralizing antibody titer in serum appeared to be more dependent on the time of collection than the severity of disease. Children who experienced a relatively mild respiratory illness because of the H5N1 virus had neutralizing antibody titers equivalent to those of severely ill adults. However, there were notable deviations from the kinetics curve. Most apparent was the lack of neutralizing antibody in a single serum taken from case patient 18 at 23 days after the estimated time of symptom onset (figure 1). This individual had systemic lupus erythematosus and was receiving steroid treatment that may have precluded an antibody response to the virus. Alternatively, it has been estimated that 13%–27% of culture-confirmed influenza infections in otherwise healthy individuals do not result in a detectable serum antibody response [14].

In this study, the presence of serum antibody to H5N1 virus has been used as evidence of infection of family and social contacts exposed to 16 confirmed H5N1-infected case patients. In some instances, interpretation of the antibody data were limited because of the lack of an acute serum sample or the lack of culture confirmation of infection. Only 1 of 6 H5-antibody–positive household contacts was symptomatic and was confirmed, by virus isolation, to be infected with H5N1 virus. The other 5 seropositive household contacts and 1 seropositive social contact were asymptomatic, suggesting limited virus replication in these individuals [13]. A significant rise in serum HI antibody in the absence of a febrile illness in individuals who were experimentally infected with human influenza A (H2N2) virus has been reported elsewhere [15].

Twelve percent of household contacts were positive for H5 antibody. This high seroprevalence among household contacts may reflect H5N1 transmission between people but may also reflect similar environmental exposures among contacts and case patients. In a stratified analysis, the increased risk of H5-antibody positivity in household contacts exposed to poultry was consistent with the results of a case-control study in which exposure to live poultry in the marketplace was identified as the primary risk factor for infection with H5N1 virus [12]. Only 1 of 3 H5-antibody–positive adult household contacts had no history of poultry exposure. This contact was the father of case patient 9, a 4-year-old boy, with whom the father played and had close physical contact, including cuddling the child. However, this individual was already seropositive 9 days after the infected child became ill, suggesting either an early response to infection or an earlier-than-estimated symptom onset in the child. Alternatively, the individual may have had prior exposure to H5N1 virus in the environment, perhaps from the same source that infected the child. The grandmother who cared for case patient 9 was also positive for H5 antibody 9 days after the onset of the child's illness. This response was consistent with her history of poultry exposure. Likewise, an adult household contact of another child, case patient 2, had been exposed to poultry in late September 1997. Because this adult, a boarder in the household, had very little contact with the case patient, it is likely that his exposure to poultry was the source of infection with H5N1 virus.

The H5-specific ELISA and Western blot test combination detected antibody in 3 children. Case patient 8 was a contact of case patient 5 and was the only antibody-positive contact from whom virus was cultured. Case patient 8 had close physical contact with case patient 5, when the latter was symptomatic. Physical contact included hugging and kissing. However, both children had also been exposed to poultry at retail stalls located close to the home of case patient 8 during the week before the onset of symptoms. Fecal swabs collected from these poultry stalls yielded influenza A (H5N1) virus (K. Shortridge, personal communication). Exposure to poultry cannot be excluded as the cause of the H5N1 infection in case patient 8; however, the timing of illness onset in relation to exposure to case patient 5 suggests that human-to-human transmission of the virus might have occurred.

The 2 other children who tested positive for H5 antibody were children of case patient 6. A third child, an infant, was seronegative. H5-specific IgG and IgM were detected in the 5-year-old child 26 days after the earliest exposure to the case patient. Although the timing of the earliest serum collection

Table 3. Characteristics of H5 antibody–positive household contacts.

Contact age (y), sex	Case patient	Relationship to case patient	Type of contact	Respiratory illness	Exposure to poultry	Serologic response: Days after exposure[a]	Neut/WB[b]	ELISA/WB[b]
41, M	2	Household boarder	No direct contact	No	Yes	22	+	
33, M	9	Father	Close physical contact	No	No	9	+	
						24	+	
58, F	9	Grandmother	Cared for while ill	No	Yes	9	+	
						24	+	
2, M[c]	5	Cousin	Play, including close physical contact	Yes	Yes	2		−
						16		+[d]
5, F	6	Daughter	Close physical contact	No	Yes	26		+
						51		+[d]
4, F	6	Daughter	Close physical contact	No	Yes	26		−
						51		+

NOTE. M, male; F, female; Neut, microneutralization assay; WB, Western blot.

[a] No. of days are based on the earliest date of exposure to case patient that was considered to be 1 day prior to onset of case patient's illness through 14 days after onset of illness.

[b] Western blots of adult's and children's sera were developed with anti–human IgG and total Ig, respectively.

[c] Case patient 8.

[d] Also positive for H5-specific IgM.

precluded documentation of seroconversion, the detection of H5-specific IgM antibody suggested recent infection. In contrast, seroconversion for H5-specific antibody was documented for the 4-year-old daughter, but this occurred >26 days after case patient 6 was first symptomatic and after the case patient had recovered and returned home. A number of poultry stalls were located near the home of case patient 6, including 2 on the ground floor of the building in which the family resided. Case patient 6 had purchased poultry several times from the stalls during the first half of November. On a subsequent inspection of these stalls, the Hong Kong Department of Health collected fecal swabs from which H5N1 virus was cultured (K. Shortridge, personal communication). Therefore, all household members potentially were exposed to infected poultry before the slaughter of poultry, which began on December 29, 42 days after the onset of symptoms for case patient 6. In contrast with the prevalence of antibody to H5N1 virus in 2 of 4 household contacts of case patient 6, none of the case patient's exposed coworkers were positive for H5 antibody. Thus exposure during social contact or the performance of office-related duties was not associated with transmission of H5N1 virus.

Members of the tour group were exposed to case patient 4, who was acutely ill during the 4-day tour. Case patient 4 was admitted to hospital on his return to Hong Kong and died 9 days later. Although all tour participants were exposed to case patient 4 in closed environments during flights or bus rides of several hours duration, only 1 of 26 tour members (4%) tested positive for antibody to H5N1 virus. Previous reports on the transmission of airborne pathogens in a commercial aircraft have associated the length of confinement and seating proximity to an infected individual with the risk of infection [16, 17]. In this study, the antibody-positive individual was not seated in close proximity (<3 rows) to case patient 4 on either flight or on any of the bus rides. Twenty-two of 26 individuals (85%), including the seropositive individual, had further social contact with the case patient during meals, by talking with him, or observing him cough.

The source of the H5N1 infection, whether human or poultry, in these seropositive individuals cannot be known with certainty. However, the time course of infection and serologic response of case patient 8 were consistent with infection resulting from exposure to case patient 5. The father of case patient 9 had no history of exposure to poultry, suggesting that the origin of infection may have been the H5N1-infected child. These 2 household contacts both had close physical contact that included hugging, kissing, or cuddling the infected individuals to whom they were exposed. The results of cohort studies in health care workers exposed and not exposed to H5N1-infected case patients suggested that limited transmission of the virus from human to human likely occurred [18]. Exposures that required close personal contact, such as bathing and changing of bed linen, were associated with seropositivity in the health care workers.

This study shows that previously healthy humans infected with influenza A (H5N1) virus mount a serum neutralizing antibody response to the avian virus with kinetics similar to

Table 4. Prevalence of H5 antibody–positive individuals in cohorts, stratified by poultry exposure.

Cohort	*n*	No. of antibody-positive individuals/total (%): Exposed to poultry	Not exposed to poultry	*P*
Household contacts	45[a]	5/24 (21)	1/21 (5)	.13
Tour group members	26	1/16 (6)	0/10 (0)	.62
Coworkers				
Exposed	23	0/9 (0)	0/14 (0)	NA
Not exposed	24	0/3 (0)	0/21 (0)	NA

NOTE. NA, not applicable.

[a] Status of poultry exposure was determined for only 45 of the 51 household contacts.

those observed in a primary response to human influenza A viruses. The presence of H5-specific antibody has been used to detect evidence of infection in individuals exposed to H5N1-infected individuals. A seropositive family member who experienced close personal contact with an H5N1-infected child and had no history of poultry exposure provided evidence that human-to-human transmission of the avian virus was likely to have occurred. However, results of the tour group and coworker cohorts indicate that, unlike human influenza A H1N1 and H3N2 viruses, the avian H5N1 viruses are not readily transmitted from person to person in a social setting, even among a seronegative population.

Acknowledgments

We thank Anthony Mounts, Joseph Bresee, and Seymour Williams for assistance with data collection; Hong Kong Hospital Authority staff for providing patient sera; Pam Uveges for assistance with data handling; Renee Black and Judy Galphin for valued technical assistance; and Hector Izurieta for review of the manuscript.

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