CHAPTER IV

RESEARCH RESULTS

4.1 Patients and contact history

The median age of the 25 case patients (11 of who were male and 14 female) was 30 years (range, 6 to 62). The patients were from both rural and urban parts of China (9 living in urban settings and 16 living in rural settings). There was one confirmed family cluster with 2 cases.

Data were analyzed for 22 of the 25 known H5N1 cases. As mentioned above, analysis considered incubation period exposure settings, including cases exposed only to sick/dead poultry (direct contact or indirect exposure <1 meter) versus those exposed only to a wet poultry market. We excluded three cases, including one without an identified exposure source, 2 in a cluster with limited person-to-person transmission reported elsewhere.

The maximum time from first exposure to illness onset was limited to 14 days for biological plausibility. For cases with exposures on multiple days, we calculated the median incubation period for each case, and then calculated the overall median of the distribution of these median incubation periods, with a range of the medians among the cases. Similarly, the minimum and maximum incubation periods for cases with exposures on multiple days was estimated by using the last or first known exposure day, respectively. The overall incubation period among H5N1 cases was

estimated by determining the median of the distribution of case's median incubation periods. Incubation periods were compared using the Wilcoxon Rank Sum Test.

Of the 22 H5N1 cases, 14 (64%) had exposure to sick/dead poultry only, and 8 (36%) had visited a wet poultry market only (Table 3). For cases with ≥2 exposure days (N=16), and for cases with a single exposure day (N = 6), the overall median incubation period was longer for cases that had visited a wet poultry market than for those who were exposed to sick/dead poultry. When all 22 cases were considered together, the incubation period also tended to be longer for those who had visited a wet poultry market. This difference was marginally significant for the median incubation period (p=0.086) and the maximum incubation period (p=0.064).

Table 2: Estimated incubation period (days) of 22 human cases of infection with avian influenza A (H5N1) virus, China

Case-patients with exposures on multiple days	Exposed to sick/dead poultry only, n=10	Exposed to wet poultry market only, n=6	p-value	Total, N=16
Overall median incubation period (range)	5 (2-9.5)	6.3 (3.5-7)	0.510	5.5 (2-9.5)
Median of minimum incubation period (range)	1 (0-5)	0 (0-2)	0.227	0.5 (0-5)
Median of maximum incubation period (range)	8.5 (4-14)	11.5 (7-14)	0.352	9 (4-14)

Case-patients with single known exposure	Exposed to sick/dead poultry only, n=4	Exposed to wet poultry market only, n=2	p-value	Total, N=6
Overall median incubation period (range)	3.5 (2-6)	8.5 (8-9)	0.064	5 (2-9)

Case-patients with single or multiple exposures	Exposed to sick/dead poultry, n=14	Exposed to wet poultry market, n=8	p-value	Total, N=22
Overall median incubation period (range)	4.5 (2-9)	7 (3.5-9)	0.086	5.5 (2-9.5)
Median of minimum incubation period (range)	1.5 (0-6)	1 (0-9)	0.729	1.5 (0-9)
Median of maximum incubation period (range)	6.5 (2-14)	9 (7-14)	0.064	8 (2-14)

4.2 Clinical time course

The median days from illness onset to the first medical consultation was 1 day (range 0-6). Medical care was most sought from lowest level of public and private health center clinics (Village/Community). Among the total of 25 case patients, 17 (68%) consulted physicians in the village or community. Compared with case patients living in urban area, more H5N1 cases in rural of China would first consult with health facilities in their village (p=0.018). No first medical consultation for rural cases patients occurred in a hospital at a level above township

Most H5N1 patients had initial symptoms of high fever (typically a temperature of more than 38°C) and an influenza-like illness with lower respiratory tract symptoms. However, 24 cases (96%) were diagnosed as upper respiratory infections when they first consulted with physicians in different level of health facilities. Case 27 from the family cluster was under medical observation after his son's death from confirmed infection and developed pneumonia the second day of his illness onset, so he was diagnosed as pneumonia when he was admitted to hospital. Otherwise, all case patients (96%) were assessed as outpatients and discharged home. Median number of days from illness onset to the first hospitalization was 6 days (rang 1-16). There was no time difference between case patients in urban and rural area. All of cases in urban area were admitted to hospitals above prefecture level compare with case patients in rural areas (p=0.003). 23 (92%) case patients were diagnosed as pneumonia when they were admitted to hospital. Case 8 and case23 had been diagnosed as tuberculosis when they were first admitted to hospitals. Up to 6 medical consultations had occurred before case patients were admitted to hospitals. Median number of days from illness onset to death or discharge was 23 (range 8-99). Urban

case patients were reach to disease outcome (died or discharged) earlier than were rural case patients (median, 13 vs. 27 days), and the difference was close to statistically significant ($P = .056$, median test). Case 23 and case 19 were discharged almost 3 months after their admission, due to complications of their illness (Table 4).

Table 3: Timeline of medical consultation and admission of 25 rural and urban H5N1 cases, China, October 2005 – December 2007

Variables	Rural cases (n=16)	Urban cases (n=9)	Total (n=25)	p value
Medical consultation and admission				
Median no. of days from illness onset to the first medical consultation (range)	1 (0-6)	1 (0-6)	1 (0-6)	0.244
Hospital or clinic level of the first medical consultation N (%)				
Provincial	0 (0)	1 (11)	1 (4)	
Prefecture	0 (0)	3 (33)	3 (12)	
County/District	0 (0)	0 (0)	1 (4)	**0.018**
Township	2 (13)	0 (0)	3 (12)	
Village/Community	14 (87)	5 (56)	17 (68)	
Diagnosis at first medical consultation N (%)				
URI	16 (100)	8 (89)	24 (96)	0.360
Pneumonia	0 (0)	1 (11)	1 (4)	
Median no. of days from illness onset to the first hospitalization (range)	6 (4-16)	4 (1-10)	6 (1-16)	0.179
Hospital level of the first admissionN (%)				
Provincial	0 (0)	3 (33)	3 (12)	
Prefecture	6 (38)	6 (67)	12 (48)	
County	7 (44)	0 (0)	7 (28)	**0.003**
Township	3 (19)	0 (0)	3 (12)	
Admission Diagnosis N (%)				
Pneumonia	14 (88)	9 (100)	23 (92)	0.520
TB	2 (12)	0 (0)	2 (8)	
Frequency of medical consultation before admission	3 (1-6)	3 (1-6)	3 (1-6)	0.884
Frequency of hospital transfer	1 (0-2)	0 (0-2)	0 (0-2)	0.226
Median number of days from illness onset to death or discharge (range)	27 (8-99)	13 (8-60)	23 (8-99)	**0.056**

4.3 Case detection and reporting

Among all case patients, the median duration from onset of symptoms until detected as PUO case was 7 days (range, 1–19 days). There was no significant difference between rural cases and urban cases. (Table 5)

Table 4: Timeline of case reporting and confirmation of 25 H5N1 cases, China, October 2005 – December 2007

Variables	Rural cases (n=16)	Urban cases (n=9)	Total (n=25)	p value
Reporting and confirmation				
Median no. of days from illness onset to the first reporting as a PUO (range)	7 (4-19)	6 (1-10)	7 (1-19)	0.422
Median no. of days from illness onset to confirmation with H5N1 (range)	8 (6-36)	7 (2-12)	8 (2-36)	0.069
Median no. of days from suspect PUO to confirmation with H5N1 (range)	1 (0-2)	2 (0-29)	1 (0-29)	0.170
Median no. of days from first medical consultation to suspect PUO (range)	5 (1-19)	4 (0-8)	5 (0-19)	0.178
Median no. of days from first admission to suspect PUO (range)	1 (0-3)	1 (0-4)	1 (0-4)	0.881

4.4 Virological testing

Research teams collected 55 specimens for virological diagnosis, including 34 (62%) from the upper respiratory tract (URT) (nasal swab, throat swab, nasopharyngeal swab, sputum) and 21 (38%) from the LRT (bronchial aspirate, endotracheal aspirate, pleural fluid, alveolar aspirate, lung tissue biopsy).

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endotracheal aspirate, pleural fluid, alveolar aspirate, lung tissue biopsy). Specimens collected within 7 days after illness onset presented high proportion be tested as positive by methods of virus RNA detection and virus isolation (P= 0.006 and P=0.013, respectively). This mean that earlier collection was associated with significantly higher rates of RNA Detection and virus isolation.

26 of 34 samples (76%) collected from upper respiratory tract were obtained by throat swab. Within the upper respiratory tract, the location of specimen collection was not associated with test positivity. However, specimens collected from lower respiratory tract may be more likely to be tested as positive by virus isolation (p=0.015). (Table 6)

91% of specimens from the LRT yielded H5N1 AIV compared to 53% from the URT. Sensitivity of reverse transcription-polymerase chain reaction detected H5N1 AIV RNA is high (89%) and overall agreement (both test as negative or positive) of test is 75% (Table 7).

Table 5: Time and location of respiratory specimens' collection associated with H5N1 virus culture positive

Potential risk factors	Virus RNA detection				Virus isolation			
	Positive n=43 (%)	Negative N=12 (%)	OR (95%CI)	p value	Positive n=37, %	Negative n=18, %	OR (95%CI)	p value
No. of days from onset illness to specimen collection								
>7 days	23 (53)	1 (8)	Ref	Ref	20 (54)	4 (22)	Ref	Ref
≤7 days	20 (47)	11 (92)	23.6 (2.5-220.3)	**0.006**	17 (46)	14 (78)	6.7 (1.6-28.5)	**0.010**
Location of specimen collection								
URT	22 (51)	2 (100)			18 (49)	16 (89)	Ref	Ref
LRT	21 (49)	0 (0)			19 (51)	2 (11)	0.1 (0.0-0.4)	**0.004**

Table 6: Evaluation of PCR for Avian Influenza A (H5N1) virus in respiratory samples.

Result by PCR	H5N1		Total
	Culture Positive	Culture Negative	
Positive	33 (60)	10 (18)	43 (78)
Negative	4 (7)	8 (15)	12 (22)
Total	37 (67)	18 (33)	55
Sensitivity (%)	89		
Specificity (%)	44		
Overall agreement (%)	75		