

CHAPTER III

RESEARCH METHODOLOGY

3.1 Research design

A cross-sectional study was conducted among all confirmed human H5N1 cases in China mainland from 2005 to 2007. The majority of information was collected by a structured questionnaire in the field as part of government public health investigations in outbreaks of H5N1 in human cases during December 2005 to June 2007. Depending on the outcome of the field investigation, additional study on time course of case detection and confirmation was undertaken to advance understanding of the epidemiological, virological, and clinical aspects of infection with the A (H5N1) virus. (Figure 6)

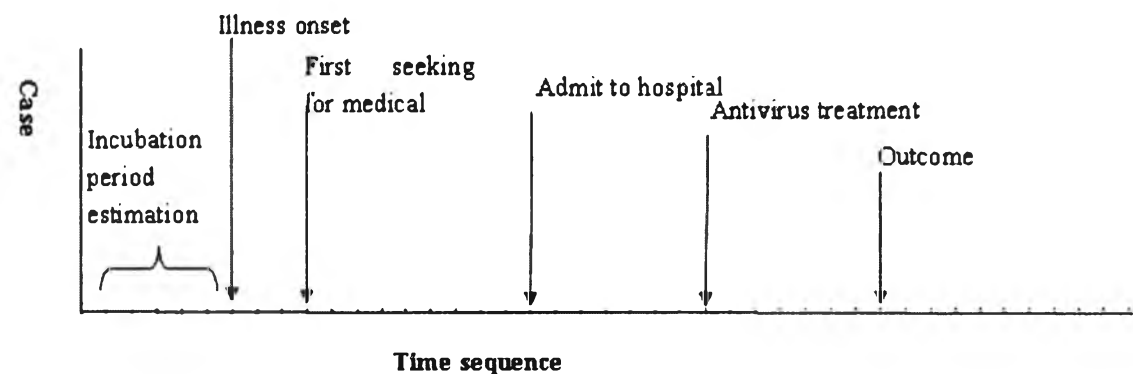


Figure 6-1: Time course 1: Timeline of clinical events in humans with avian Influenza A (H5N1) infection, China, 2005-2007

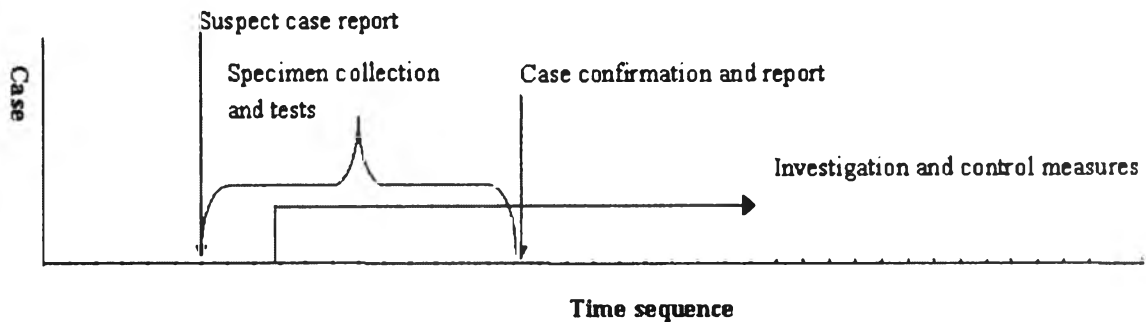


Figure 6-2: Timeline of disease confirmation and reporting in humans
with avian influenza A (H5N1) infection, China, 2005-2007

3.2 Research setting

25 cases were located in 13 provinces of China, (including Hunan, Liaoning, Anhui, Fujian, Guangdong, Shanghai, Xinjiang, Guangxi, Hubei, Jiangxi, Sichuan, Zhejiang and Jiangsu). Case patients' villages or communities were visited by investigation teams. There were 9 cases identified in 8 cities of 7 provinces (urban cases); each city had an average population of 8.3 million and the cities in which cases occurred were at least 112 km away from each other. (H et al., 2007).

3.3 Research population

Study subjects included laboratory confirmed avian influenza A (H5N1) human cases, or the proxies of decedent cases and pediatric cases, which were detected by National Surveillance System since 2005.

3.4 Data source

With approval from leaders of China CDC and Provincial CDCs, staffs from both the local and China CDCs interviewed all H5N1 cases' relatives to develop timelines of events.

3.5 Data collection

Epidemiologic and clinical data on H5N1 cases were collected from field investigation interviews and review of hospital medical records. Details are given below.

Questionnaire Survey

A structured questionnaire was used to collect information from case patients or their proxies. Based on field investigation, medical records of each cases patients and the information collected from other interviewees related to the treatment and hospitalization had been used for this study to identify or confirm the contact with HCWs and detect risk factors as follows:

- Age, sex, date of onset, and outcome (died or survived) of disease
- Timeline of consultation in different health facilities
- Process of case detection and report through the surveillance system

Staff from the China CDC interviewed each person who was ill due to infection from the H5N1 virus. An adult member of the household as proxy will be interviewed if the case patient was less than 12 years old or passed away had died. Information was collected to verify the cases' reported exposure histories for the 2

weeks prior to symptoms onset and medical histories from date of illness onset to the case recovered or to the day of death.

3.6 Laboratory assay

Blood was obtained for bacterial culture from all cases upon hospital admission. Respiratory specimens, (nasal, throat, and nasopharyngeal swabs; nasopharyngeal aspirates; saliva; and bronchial aspirates) were collected from case-patients during hospitalization. RNA was extracted from respiratory specimens using the Qiagen RNeasy kit according to the manufacturer's protocol and tested by conventional RT-PCR using primers and protocol as recommended by WHO17 and by real-time RT-PCR using H5N1-specific primers and probes from the Centers for Disease Prevention and Control, Atlanta.¹⁸ These assays were performed in biosafety level (BSL) 2 facilities at provincial CDCs and the National Influenza Center (NIC) of China CDC. Respiratory specimens were inoculated in amniotic and/or allantoic cavities of special pathogen free (SPF) embryonated chicken eggs for viral isolation in enhanced BSL 3 facilities at the NIC.

H5N1 antibody testing was performed on sera at the NIC by microneutralization (MN) assay in a BSL-3 laboratory,²⁰ and modified hemagglutination-inhibition (HI) assay using horse red blood cells in BSL-2 conditions, as previously described.²¹ Antigens for the assays were selected to match the characteristics of H5N1 viruses circulating at the same times and locations where the cases occurred. Sera were tested in duplicate by two separate microneutralization assays conducted on different days.

3.7 Training for investigators and pre-test of questionnaire

A one-week training session was held at China CDC, Beijing, for all researchers and investigators to explain the purpose of the study, familiarize them with the data abstraction form, and answer questions or concerns related to the evaluation. The questionnaire was reviewed by Field Epidemiology Training Program advisors for clarification.

3.8 Data base and analysis

Part of the overall data set was transferred to a new database as following sequence by research group through Epidata (version 3.02)

1. Check questionnaire, and corrects any errors, returning to verify data with the respondent if necessary
2. Double entry of data into the computer; the two data files were compared to find any typing errors
3. Data export for analysis.

The database for this research will be managed in Excel and imported to SPSS for further analysis

Data analysis

1. Descriptive statistics to summarize important features of numerical data and determine distribution of variables
 - Describe the central tendency and variability of data
 - Looking at variables one at a time: mean, median, range, proportion

2. Measure associations among two or variables to estimate pattern and strength of associations among variables. All statistical tests will be two-sided. A p-value of < 0.05 will be considered statistically significant.

3. Dependent variables and independent variables

Dependent variables: Outcome of disease, time interval between date of illness onset and admit to hospital, time interval of illness onset and antiviral treatment, time interval of illness onset and disease confirmation, time interval of disease confirmation and case report.

Independent variables: Age, gender, types of specimen.

Data arrangement tables (Table 1-1 and table 1-2)

4. Data were analyzed using SPSS (version 13.0, SPSS Inc, Chicago, IL, USA).

- P-Plot function in SPSS 13.0 was used for testing normal distribution of data from time between exposures to illness onset. Distribution of variables were described by mean \pm SD (rang) if the data were normal distribution.
- For categorical variables, frequencies for rural and urban groups were compared using Fisher's exact test.
- Median and range values were calculated for continuous variables, and rural and urban groups were compared using Wilcoxon rank sum test.

All statistical tests were two-sided. The significance level was set at $\alpha = 0.05$

Table 1-1: Data management of timeline study for AI human cases in China, 2005-2007

	Time Frequency	D1	D2	D3	D4	D5	D6	D7
Patient 1	Clinical timeline	Onset	Private clinic	OPD	Admit to hospital		Antivirus treatment	Outcome
	Test Timeline				S1	T1, S2	T2, S3, S4, S5	T3, T4, T5
	Report Timeline			Suspect case report				Case report
Patient 2	Clinical timeline	Onset	OPD	OPD	OPD	OPD	Admit to hospital	Outcome
	Test Timeline		S1	T1	S2	T2	S3, S4, T3, T4	
	Report Timeline						Case report	

Table 1-2: Data analyze plan of timeline study for AI human cases in China, 2005-2007

No.	Variable name	Code	Definition	Analysis plan and Interpretations
1	Age		Case patient' age at the moment of illness onset	Median,
2	Sex	M: Male F: Female	Case patient' sex at moment of illness onset	Proportion
3	Resident	R: Rural U: Urban	Case patient' dwelling place at the moment of illness onset	Proportion, Divided cases into 2 groups by resident (urban and rural) and analyze the distributions of No. 5, 7, 9, 12, 15, 17, 18, 19, 20 by groups
4	Date of first Exposures	month/day/year	On that date, case patient the first time had exposed one or more possible contaminate H5N1 sources before illness onset	Calculate intervals with No. 6, 8, 9, 16; Statistical testing of these intervals by age and resident.

Table 1-2: (Continual) Data analyze plan of timeline study for AI human cases in China, 2005-2007

No.	Variable name	Code	Definition	Analysis plan and Interpretations
5	Type of first Exposures	1: Poultry related exposures 2: Wet poultry market related exposures 3: AI confirmed case exposure 5: Unclear	1: Poultry related exposures- Direct or indirect contacted with sick or dead poultry either confirmed H5N1 infection or suspected death; direct or indirect contacted with health appeared poultry; consume poultry food 2: Wet poultry market related exposures- Have ever visited one or more market with live poultry selling and/or slaughtering 5: Unclear- Lack of any of above	Proportion and statistical testing by resident
6	Date of last Exposures	month/day/year	On that date, case patient the last time had exposed one or more possible contaminate H5N1 sources before illness onset	Calculate intervals with No. 8, 9, 16; Statistical testing of these intervals by age and resident.

Table 1-2: (Continual) Data analyze plan of timeline study for AI human cases in China, 2005-2007

No.	Variable name	Code	Definition	Analysis plan and Interpretations
7	Type of last Exposures	Refer to No. 5	Refer to No.5	Proportion
8	Date of illness onset	month/day/year	Date of first onset of symptoms	Calculate intervals with 9, 12, 16, 17, 18, and 21. Statistical test by age and resident
9	Date of first medical consultation	month/day/year	Date of the first time that case patient went to any level of health facilities to seek treatment from a physician	Calculate intervals with 12, 16, 17, 18, and 21. Statistical test by age and resident
10	Name of first Diagnosis		The specific diagnosis which has been given by a physician when case patient seek for medical consultation the first time	Description

Table 1-2: (Continual) Data analyze plan of timeline study for AI human cases in China, 2005-2007

No.	Variable name	Code	Definition	Analysis plan and Interpretations
11	Level of health facilities of first consultation	1: Provincial level 2: City level 3: County level (including hospital of enterprise) 4: Township level, community health care centers 5: Village level, private clinics	Based on the structure of Chinese health system and field investigation.	Proportion and description. Statistical test by 3, 15, 21, 24, and 25
12	Date of first hospitalization	month/day/year	The first time that case patient had been admit to any level of health facilities	Calculate intervals with 16, 17, 18, and 21. Statistical test by age and resident

Table 1-2: (Continual) Data analyze plan of timeline study for AI human cases in China, 2005-2007

No.	Variable name	Code	Definition	Analysis plan and Interpretations
13	Admit Diagnosis		The specific diagnosis which has been given by a physician when case patient had been admit to a health facilities	Description
14	Level of health facilities of admit	Refer to No. 11	Refer to No. 11	Proportion and description. Statistical test by 3, 15, 21, 24, and 25
15	Outcome	1: alive 2: death		Proportion
16	Date of Outcome	month/day/year		
17	Date of Report PUO	month/day/year	Base on national real-time internet surveillance system, to record the date of case patient had been reported by any level of health facilities or CDCs	Calculate intervals with 21. Statistical testing by 22, 23, 24, 25

Table 1-2: (Continual) Data analyze plan of timeline study for AI human cases in China, 2005-2007

No.	Variable name	Code	Definition	Analysis plan and Interpretations
18	Date of Disease confirmation	month/day/year	1. Date of first test of H5 as a positive result from CDCs in province 2. Date of paralleled laboratory tests in national level to confirm H5N1 infection	Calculate intervals with 21. Statistical testing by 22, 23, 24, 25
19	Times of seeking treatment before admit		The times of case patient had visited the health facilities before admit	Statistical testing by 11 and resident
20	Times of Transfer between hospitals		The times of case patient had been transferred between hospitals after admit	Statistical testing by 14 and resident

Table 1-2: (Continual) Data analyze plan of timeline study for AI human cases in China, 2005-2007

No.	Variable name	Code	Definition	Analysis plan and Interpretations
21	Date of specimen collection	month/day/year	Based on official report of China CDC	
22	URT	1: Yes 2: No	Any specimens as the following based on WHO definition: Nasal swab, throat swab, nasopharyngeal swab, saliva	Proportion
23	LRT	1: Yes 2: No	Any specimens as the following based on WHO definition: Bronchial aspirate, endotracheal aspirate, pleural fluid, alveolar aspirate, lung tissue biopsy	Proportion
24	RT-PCR, Real time PCR	1: positive 2: negative 3: Undo	Refer to WHO standard definition	Proportion

Table 1-2: (Continual) Data analyze plan of timeline study for AI human cases in China, 2005-2007

No.	Variable name	Code	Definition	Analysis plan and Interpretations
25	Virus Culture	1: positive 2: negative 3: Undo	Refer to WHO standard definition	Proportion

3.9 Ethical review

The research is a part of public health emergency response and is scientifically valid. Oral informed consent for specimen collection was obtained.