CHAPTER II

LITERATURE REVIEW

The literature review has been divided into 5 parts 1) Avian influenza A (H5N1) surveillance system 2) Epidemiology and clinical characteristics of human infection with H5N1 3) Risk factors of human infect with H5N1 4) H5N1 virus attachment and laboratory tests. 5) WHO guidelines on avian in H5N1 cases report. No literature with the specific objective of analyzing the time course of human H5N1 infection was found.

2.1 Avian influenza A (H5N1) surveillance system

For close global monitoring of the situation and coordination of the global response, the World Health Organization (WHO) is recommending enhanced surveillance for influenza A/H5 until further notice. General objectives are to monitor the spread of influenza A/H5 viruses in human and animal populations in order to assess the global trend of the disease, the public health risk it poses, and its pandemic potential, also to trigger public health actions for pandemic preparedness as specified in the Influenza pandemic preparedness plan (WHO, 2007). H5N1 case-finding has not focused on the identification of asymptomatic or mild cases of H5N1 virus infection (Yuen et al., 1998; Bridges et al., 2002) which would likely decrease the overall case-fatality proportion, although one limited clade 1 H5N1 virus seroprevalence study published to date did not identify any asymptomatic infections

(Vong et al., 2006). Four clinically mild cases of H5N1 virus infection in Indonesia in 2005–2006 were identified during field investigations of severely ill index case patients in 3 clusters and would have been missed by hospital-based surveillance for severe respiratory disease (Kandun et al., 2006).

Hong Kong (Chan 2002; Ho & Parker, 2006) Since the 1997 H5N1 outbreak in HKSAR, the HKSAR government has launched new policies focus on prevention and early detection of reemergence of an epidemic of infection with H5N1 or a similar cross-species influenza virus. Before poultry may be imported into Hong Kong, chickens must be segregated in designated farms in mainland China for 5 days and tested for H5 infection. Implementation of a policy to segregate chickens from waterfowl at all levels of the industry, from import to retail, has been recommended. Poultry surveillance for H5N1 in farms has been introduced, as have new licensing conditions covering hygiene and management practices. All flocks must be serologically tested during the growing period. With these surveillance procedures and policies, it is hoped that reassortment of influenza virus genes between avian species can be minimized and that cross-species transmission of new influenza virus strains from birds to humans can be prevented.

The Centre for Health Protection CHP has been set up to optimize and develop the capacity of existing healthcare providers through expanding the disease surveillance network with the aid of information technology, setting up a structured epidemiology training program, enhancing clinical diagnostic laboratory services, developing applied research and preparing emergency response plans. Its work is supported by multidisciplinary programs on specific diseases and health threats, with professional expertise drawn from various sectors of the community. Enhancement of

communication and liaison with the community are seen as vital to public health protection and are a major component of the CHPs activities. The framework and objectives that have been developed for managing an influenza pandemic in Hong Kong are in line with the latest WHO guidelines. The plan incorporates the key strategies and policies as suggested in the 1999 and 2005 guidelines, and the new WHO phases have been simplified for easy public understanding into three response levels, namely alert, serious, and emergency. The current framework is based on scenarios relevant to Hong Kong. The plan takes account of each of the key areas identified by WHO: surveillance, investigation and control measures, laboratory support, infection control measures, provision of essential medical services, antiviral stockpiling, vaccination, port health measures and communication. It identifies major activities/measures to be undertaken in relation to each of these, in normal times and at times requiring alert, serious and emergency response levels.

Thailand (Areechokchai et al., 2006; Olsen et al., 2006)**:** As a newly industilized country, Thailand has substantial funding and infrastructure at its disposal, and it has made use of additional resources from CDC and WHO for local and regional surveillance and training efforts. For example, it has trained more than 1000 surveillance and rapid response teams in all 76 provinces, and has enlisted some 800 000 village health volunteers to assist in the identification and reporting of possible H5N1 cases in people and poultry. Since 2001, the Thailand Ministry of Public Health and the US Centers for Disease Control and Prevention (CDC) have collaborated to implement active, population based surveillance for severe pneumonia requiring hospital admission in two provinces in rural Thailand. Standard case definitions, routine radiographic confirmation with standard interpretations from a

panel of radiologists blinded to clinical information produce reliable data on incidence, which range from a measured incidence of 177 to a maximum estimate of 580 cases per 100 000 people per year. The Thailand Ministry of Public Health reported a cumulative total of 5354 influenza-like illness or pneumonia cases that were investigated in 72 of 76 provinces between Jan 1 and Oct 26, 2006—an average of 535 cases per month. Reporting was lower in provinces deemed unaffected and during months when H5N1 virus among poultry was not observed. Among these suspected human cases, only three had laboratory-confirmed avian influenza A (H5N1). Many more people have had confirmed human influenza infection, especially during July, a peak month for human influenza in Thailand, as recently shown by the enhanced surveillance system. These data indicate both a low rate of true cases and an active surveillance system with routine reporting. Under Thailand's nationwide avian influenza A (H5N1) surveillance system, which BoE initiated in December 2003, any patient admitted to a health-care facility with pneumonia or influenza-like illness who has a history of exposure to poultry is reported to BoE. For this study, a case was defined as H5N1 illness occurring in a person who had received a diagnosis of pneumonia or influenza-like illness and who had either a positive viral culture for H5N1 virus or confirmation of H5 strain by real-time reverse transcription-polymerase chain reaction (RT-PCR).

Vietnam (Dinh et al., 2006; Nguyen et al., 2007): The National Communicable Disease Surveillance of influenza-like-illness (ILI) has been conducted in Vietnam, but solely based on clinical case reports. Ten outpatient private clinics and two hospitals (Bach Mai and National Institute of Pediatrics), mainly pediatrics, participated in the study. ILI was defined on the basis of sudden onset of fever (>37.8 _C) and any

signs or symptoms of acute respiratory infection such as coughing, a sore throat, a runny, or stuffy nose. Participants were asked for oral informed consent at the time of enrolment. Patient demographic details such as name, sex, age, address, and clinical symptoms, were recorded at hospitals and clinics upon collection, and then the samples and data were sent to the Respiratory Virus Section, Virology Department in National Institute of Hygiene and Epidemiology (NIHE), which is a governmental institution in Hanoi affiliated to the Ministry of Health in Vietnam.

Case-patients were identified from persons hospitalized with an acute respiratory infection considered by clinicians, on the basis of clinical and epidemiologic findings, to have a suspected case of H5N1 infection. Clinicians did not use a systematic case definition or screening protocol to identify patients eligible for testing for H5N1 infection. Throat swabs or tracheal aspirate samples were sent to the National Institute of Hygiene and Epidemiology in Hanoi or to the Pasteur Institute in Ho Chi Minh City for reverse transcription (RT) PCR and viral isolation. When possible, samples with positive results for influenza A/H5 were sent to a World Health Organization (WHO) reference laboratory for confirmatory diagnosis.

Indonesia (Wibisono et al., 2006; Sedyaningsih et al., 2007)**:** Highly pathogenic avian influenza A (H5N1) virus was detected in domestic poultry in Indonesia beginning in 2003 and is now widespread among backyard poultry flocks in many provinces. Ill patients who met the World Health Organization (WHO) case definition for a suspected case of H5N1 virus infection were reported to the Indonesia Ministry of Health. If clinically stable, patients with suspected H5N1 virus infection were transferred to 1 of 44 designated H5N1 referral hospitals throughout Indonesia.

Available clinical specimens were obtained from patients with suspected H5N1 virus infection during hospitalization for testing at laboratories in Jakarta.

Azerbaijan (Gilsdorf et al., 2006) Following the appearance of influenza A/H5 virus infection in several wild and domestic bird species in the Republic of Azerbaijan in February 2006, two clusters of potential human avian influenza due to A/H5N1 (HAI) cases were detected and reported by the Ministry of Health (MoH) to the WHO Regional Office for Europe during the first two weeks of March 2006. On 15 March 2006, further to a request for assistance by the MoH. In accordance with national ministerial decrees issued early in 2006, district chief doctors implemented reporting of cases where avian influenza A (H5N1) virus infection was suspected from the local doctors to the MoH, those healthcare corkers were also informed and trained healthcare workers on how to detect and report such cases. All reported cases were investigated at district level and, after reporting to the central level, also by the MoH-WHO response team.

Since early February 2006, the general public was also informed, through social mobilization campaigns (e.g. distribution of posters, school lessons) at district and national level, about the risk of exposure to and mode of transmission of avian influenza A (H5N1) virus, symptoms of AI, and was invited to seek medical care if suggestive symptoms developed.

2.2 Epidemiology and clinical characteristics of human infection with avian influenza A (H5N1)

Influenza pandemics are naturally occurring large-scale epidemics of a virulent strain of influenza A virus that causes a global outbreak of serious respiratory

illness. Due to the uniqueness of the strain, the virus can spread rapidly, causing significant morbidity and mortality significantly, above yearly epidemic proportions. Pandemics tend to occur at 20–40 year intervals. Three were recorded in the twentieth century: 1918, 1957, and 1968. (Hatchett et al., 2007)Sporadic transmission of avian influenza A (H5N1) during the 2004-2006 Asian epizoonotic have prompted concerns on the next pandemic(Chang et al., 2006). There are two features in the current avian influenza A (H5N1) outbreaks: the overwhelming concentration in previously healthy young adults and the very high mortality rate (Stohr, 2005).

The first association of avian influenza A (H5N1) with clinical respiratory disease occurred in Hong Kong in 1997, when 18 human cases occurred during a poultry outbreak of HIPAI in live-bird markets (Yue et al., 1998). This outbreak was associated with a high mortality rate (33 percent), a high incidence of pneumonia (61 percent), and a high rate of intensive care (51 percent).

All virus genes were of avian origin, suggesting that H5N1 had jumped the species barrier without adaptation (Bender et al., 1999). Serologic surveillance revealed little evidence of human-to-human transmission, and no further cases were reported following mass culling of poultry (Katz et al., 1999; Bridges, 2002; Webster & Goverkova, 2006). An H5 seroprevalence rate of up to 10 percent has been observed in asymptomatic poultry workers in Hong Kong.

In 2003, H5N1 reemerged in humans when two culture-confirmed cases occurred in a family group returning to Hong Kong from China (Peiris et al., 2004). A younger family member also died of a respiratory illness, but the etiology was undetermined.

Despite culling of domestic and agricultural flocks, outbreaks in multiple provinces were reported. The first fatal human cases, outside southeastern Asia, were detected among Turkish poultry farmers and in young children with poultry exposure in early 2006(Oner et al., 2006).

Since February 2006, rapid geographical spread of H5N1 virus in wild and domestic birds has been reported in Iraq, Nigeria, Azerbaijan, Bulgaria, Greece, Italy, Slovenia, Iran, Austria, Germany, Egypt, India, France and Israel. Of particular note, the disease has now been isolated in migratory wild birds and poultry farm outbreaks in several European Union countries suggesting multiple introductions.

Since its emergence in humans in 1997, AIV (H5N1) has undergone antigenic changes (eg, antigenic drift) (Guan et al., 2004). Sequencing and antigenic characterization of the hemagglutinin genes in the currently circulating H5N1 viruses reveal significant antigenic differences between those isolated from humans in 1997 and subsequent years of 2003 through 2006. H5N1 viruses can be divided into clade 1 and clade 2 viruses; the latter can be further subdivided into three subclades.

In addition to infecting poultry and humans, H5N1 virus appears to have extended its host range into felids. During the 2003-2005 H5N1 outbreaks, there have been reports of fatal infection in domestic cats, a finding that has been confirmed after experimental inoculation (Kuiken et al., 2004). Infection can be acquired horizontally from other cats or by feeding on infected poultry or wild birds. Furthermore, H5N1 virus caused severe pneumonia in tigers and leopards at the Bangkok zoo, which fed on infected poultry carcasses (Keawcharoen et al., 2004). Several domestic cats and a stone marten died of avian H5N1 during an outbreak of lethal avian H5N1 among

swans on a nature reserve in Germany; it is presumed that they fed on the dead birds. There are no reports of cats transmitting the virus to humans.

Potential modes of transmission of H5N1 include oral ingestion of contaminated water during swimming; direct intranasal or conjunctival inoculation during exposure to water; contamination of hands from infected families and exposure to untreated poultry feces used as fertilizer (WHO 2005). One patient from Indonesia had no known contact with poultry, but did use poultry feces, which tested positive for H5N1, in her garden (Kandun et al., 2006).

In the 1997 H5N1 outbreak in Hong Kong, eight of 18 affected patients were less than 12 years of age, and all but one had relatively mild disease. The most severe infections occurred in those older than 12 years of age. In a description of 12 older patients, the presenting features included fever (100 percent), upper respiratory tract symptoms (67 percent), pneumonia (58 percent), and gastrointestinal symptoms (50 percent).

During the 2004-2006 outbreaks in Asia, the majority of patients were less than 25 years of age. In a report of 10 Vietnamese and five Thai patients, all patients presented with fever and lower respiratory symptoms (including pneumonia). All of these patients developed respiratory distress syndrome and died between six and 29 days after presentation.

In a report from Vietnam, two children from the same family presented with diarrhea and encephalopathy without any sign of respiratory compromise and later died. The presence of H5N1 infection was later established by isolation of the virus from cerebrospinal fluid, as well as from fecal, throat, and serum specimens. The

capacity of H5N1 to cause systemic illness, including central nervous system involvement, has also been demonstrated in animals.

Respiratory symptoms may be accompanied by gastrointestinal symptoms, headache, myalgia, sore throat, rhinorrhea or uncommonly conjunctivitis or bleeding gums. Diarrhea is a prominent presenting symptom, along with respiratory distress, in several case series, particularly in association with clade 1 H5N1 infection.

The incubation period of avian influenza A (H5N1) may be longer than for other known human influenzas. In 1997, most cases occurred within two to four days after exposure; recent reports indicate similar intervals but with ranges of up to eight days. The case to case intervals in household clusters have generally been 2 to 5 days, but the unrecognized exposure to infected animals or environmental sources (Beigel et al., 2005). A research in Turkey showed that The mean (±SD) time between the last known exposure to ill or dead poultry and the onset of the illness was 5.0±1.3 days (range, 4 to 7) (Oner et al., 2006). The estimated time between the exposure to poultry and the onset of illness suggests an incubation period of two to four days (Tran et al., 2004).

2.3 Risk factors for human infection with H5N1

Human influenza is transmitted by inhalation of infectious droplets and droplet nuclei, by direct contact, and possibly by indirect contact. For human avian influenza A (H5N1) infections, evidence suggests bird-to-human, possibly environment-to-human, and limited, nonsustained human-to-human transmission (WHO 2005; Kandun et al., 2006).

Of great concern are the increasing numbers of sporadic avian-to-human transmission of different subtypes of avian influenza A (H5N1) reported in Asia, the Netherlands, and British Columbia during the last few years (Trran et al., 2004; Ungchusak 2005). Most cases of human infection have involved close contact with infected poultry, especially ill or dying chickens. In 1997, 18 cases of avian influenza A (H5N1) infection were identified in Hong Kong. A case-control study of 15 of these hospitalized patients determined that exposure to live poultry in the week before illness was significantly associated with disease (Mounts, 1999).

To evaluate the potential for avian-to-human transmission of H5N1, a cohort study was conducted among 293 Hong Kong government workers who participated in the animal culling program and in 1525 poultry workers (Bridges et al., 2002). Serologic testing demonstrated that three percent of government workers and 10 percent of poultry workers had anti-H5 antibody without evidence of disease.

Limited person-to-person transmission of H5N1 virus infection has been suggested by clusters of cases of avian influenza A (H5N1) within families. In one cluster in Thailand, the index patient, an 11-year old girl who had exposure to poultry, and her mother who provided nursing care, both died with pneumonia. The girl's aunt, who had close contact with the girl, became ill with respiratory symptoms. She survived after treatment with oseltamivir. H5N1 infection was confirmed in the mother and aunt. Although no tissue samples were available for the child, her clinical presentation was consistent with H5N1 infection. Limited person-to-person H5N1 transmission may have occurred among two clusters of patients identified in Indonesia who had no known contact with poultry or other animals. In one cluster, all three family members shared a bed after the onset of illness in one individual.

However, none of the 173 contacts (household members, neighbors, health care workers, coworkers) became ill.

In Indonesia, 54 cases of H5N1 virus infection were identified from July 2005 through June 2006. More than one-third of the cases occurred in seven clusters of family members (Sedyaningsih et al., 2007). Whether case clustering was due to common source poultry exposure, limited person-to-person transmission, or genetic host susceptibility, is unclear.

In the above clusters, transmission may have occurred through close physical contact; there is no evidence of human-to-human transmission of avian influenza A (H5N1) via small particle aerosols (Hayden & Croisies, 2005). Serologic surveys in Vietnam and Thailand have not found evidence of asymptomatic infections among contacts of active cases and nosocomial transmission to health care workers has not been documented (WHO, 2005).

2.4 Laboratory tests for H5N1 virus

Specimen collection: Standard and appropriate barrier precautions should always be taken when collecting specimens from patients. Specimens should be obtained as soon as possible after the onset of symptoms. In general, a nasopharyngeal swab or aspirate is considered the preferred specimen for seasonal influenza testing; however, recent data suggest that oropharyngeal and lower respiratory tract specimens (i.e., sputa and bronchoalveolar lavage fluid) are superior to nasopharyngeal specimens for the detection of avian influenza A (H5N1) infection in humans (Beigel et al., 2005). Specimens from multiple sites may yield the best results. In cases of atypical presentations, such as gastroenteritis and encephalopathy, stool and

cerebrospinal fluid specimens, respectively, are advised. Swabs and transport media intended for bacteriologic testing are not suitable for influenza testing. In addition, swabs with calcium alginate or cotton tips and wooden shafts are not recommended. Swabs for influenza testing should have a dacron tip and an aluminum or plastic shaft.

Specimen storage and transportation: Specimens should be collected in the appropriate viral transport medium and shipped immediately to the testing laboratory (on ice, if possible) in accordance with regulations of the Transportation of Dangerous Goods Act. Specimens may be refrigerated at 4°C (± 2°C) for up to 48–72 hours; after that, specimens should be frozen at –70°C.

Specimen testing: Rapid antigen testing is not currently recommended for the detection of avian influenza A (H5N1) (WHO, 2005). Confirmatory testing and sub typing must be performed by molecular methods (e.g., reverse transcriptase polymerase chain reaction), virus culture or both. Culture of this high-risk pathogen is restricted to certified containment level 3 facilities. All specimens that test positive for avian influenza A (H5N1) must be confirmed by the National Influenza Center.

One study demonstrates the attachment of H5N1 virus to the human low respiratory tract (LRT) and identifies cat and ferret as the most suitable animal models for human H5N1 viral pneumonia, on the basis of the similarity of viral attachment pattern.(van Riel, Munster et al. 2006). This may explain a higher yield H5N1 AIV from clinical LRT specimens from human infections with H5N1 in China. A recently study reviled that viral genomic sequences were detected in the intestinal mucosa. Thus, in addition to the lungs, AIV (H5N1) infects the trachea and disseminates to other organs including the brain. The virus could also be transmitted from mother to fetus across the placenta.(Gu et al., 2007)

Quick detection for early diagnosis of suspected cases of AIV (H5N1) infection is crucial for preventative measures and symptomatic treatment. In one report form Zhejiang Province, China. RNA of AIV (H5N1) was detected from the tracheal aspirate specimen of a suspected human case by RT-PCR or real-time RT-PCR. The antiserum titers, 11 days and 19 days after patient's onset, were determined as 1:320 and 1:640, respectively, by HI assays. The H5N1 virus was also isolated from the tracheal aspirate specimen and was designated as A/Zhejiang/16/06 (H5N1). The first human case infected by AIV (H5N1) was confirmed by both etiologic and serologic diagnoses.(Yan et al.,. 2007)

2.5 WHO guidelines on avian influenza A (H5N1) cases report

1. Clinical management of human infection with avian influenza A (H5N1) virus [http://www.who.int/csr/disease/avian_influenza/guidelines/ClinicalManagement07.pdf]
2. WHO guidelines for investigation of human cases of avian influenza A (H5N1) [http://www.who.int/csr/resources/publications/influenza/WHO_CDS_EPR_GIP_2006_4r1.pdf]
    - Purpose and scope of the document
    - Objectives for investigations of human cases of A(H5N1)
    - Initiating an investigation
    - Key steps for investigation of human cases of A(H5N1)

3. Recommendations and laboratory procedures for detection of avian influenza A (H5N1) virus in specimens from suspected human cases. [http://www.who.int/entity/csr/disease/avian_influenza/guidelines/RecAIlabtestsAug07.pdf]
   - General information and intended use of this document
   - Specimen collection and handling
   - Laboratory requirements
   - Confirmation of results
   - Available laboratory techniques for detection of influenza A viruses in humans
   - Serological identification of antibodies against avian influenza A(H5N1) viruses
4. WHO guidelines for global surveillance of influenza A/H5 [http://www.who.int/entity/csr/disease/avian_influenza/guidelines/globalsurveillance.pdf]

The guidelines aimed at monitoring the spread of H5N1 infection in human and animal populations include the definition of a laboratory-confirmed case of human H5N1 infection, and describe procedures for reporting cases to WHO. The guidelines also provide recommended procedures to monitor changes in transmission patterns and to detect potential human-to-human transmission.

5. WHO case definitions for human infections with avian influenza A (H5N1) virus. [http://www.who.int/csr/disease/avian_influenza/guidelines/case_definition2006_08_29/en/index.html]

Prompt and accurate reporting of H5N1 influenza cases to WHO is the cornerstone for monitoring both the global evolution of this disease and the

corresponding risk that a pandemic virus might emerge. In collaboration with several partners, WHO has developed standardized case definitions to facilitate:

- Reporting and classification of human cases of H5N1 infection by national and international health authorities.
- Standardization of language for communication purposes.
- Comparability of data across time and geographical areas.

6. WHO Rapid Advice Guidelines on pharmacological management of humans infected with avian influenza A (H5N1) virus

The incubation period for H5N1 in people may be longer than the same period for seasonal influenza, which is around two to three days. Current data for H5N1 virus infection suggests a similar incubation period but ranging up to eight day and rarely longer (periods as long as 17 days have been reported). However, the possibility of multiple exposures to the H5N1 virus makes it difficult to define the incubation period precisely. WHO currently recommends that an H5N1 incubation period of seven days be used for field investigations and the monitoring of patient contacts