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Note

Influenza A (H5N1) in Hong Kong: an overview

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Abstract

Worldwide pandemics of human influenza virus caused extensive morbidity and mortality around the world had been documented in the 20th century. However, the mechanisms involved in the emergence of novel influenza virus and the epidemiological factors leading to pandemics are unpredictable. Southern China is postulated as the epicentre of influenza epidemics due to its agricultural-based communities and high population density. Pandemic influenza viruses are thought to arise from avian viruses through genetic reassortment among influenza viruses.

An influenza virus (H5N1) known to infect only birds previously was found to infect human causing disease and death in Hong Knog in 1997 and the outbreak involved 18 patients with six deaths. Prior to the human outbreak, the H5N1 virus was found to cause extensive death in chickens in three farms in Hong Kong. The significance of this outbreak raised worldwide concern on the possibilities that such an influenza virus may become the next influenza pandemic strain. Investigations were initiated to find the source of the virus. In addition the extend of spread in individuals in contact with the index case and infected poultry was studied by H5-specific serology. Results demonstrated that individuals in close contact with the index case or with exposure to poultry were at risk of being infected. Out of the 18 cases of human infection, eleven had severe infection with symptoms of pneumonia and multi-organ failure. All severe cases presented with lower respiratory infection and lymphopenia and six eventually died. Case-fatality ratio was high among patients over 12 years of age (five out of nine).

Control measures aimed at reducing exposure of human to potential H5-positive poultry were instituted which included culling of all poultry in Hong Kong, the segregation of water fowls and chicken, and the introduction of import control measures for chickens. Such measures had successfully controlled the outbreak and continuous surveillance of the poultry in Hong Kong of H5N1 infection is maintained to minimize future human exposure.

Keywords: Avian influenza virus; Human infection; Epidemiology

1. Introduction

An influenza virus known to infect only avian species previously was found to infect humans, causing disease and death in Hong Kong in 1997. This incidence raised worldwide concern on the possibilities that such an influenza virus might become the next influenza pandemic strain. The first such occurrence was found in a 3-year-old child who was suffering from respiratory tract infection and eventually died in May 1997. The previously healthy child was hospitalized with symptoms of fever, sore throat and abdominal pain on 16 May 1997 and presented with a rapid deterioration of clinical conditions. The child eventually died of Reye's syndrome, acute respiratory distress syndrome, pneumothorax and liver and renal failure. During the course of clinical investigation, an atypical influenza A virus was isolated from the tracheal aspirate of the patient collected on 19 May 1997 by the Government Virus Unit of the Department of Health (National Centre for Influenza Surveillance in Hong Kong). The influenza virus was submitted to the WHO collaborating centres for influenza and other centres for further typing and confirmation. The virus was subsequently identified to be a type A avian influenza virus of subtype H5N1 by the National Influenza Centre, Rotterdam, The Netherlands; the National Institute for Medical Research, London, UK and the Center for Disease Control and Prevention, USA, in August 1997. Prior to this human case of influenza A H5N1 infection, H5N1 virus had been isolated from outbreaks of influenza in chicken farms in Hong Kong in March 1997. Since this initial human case, 17 additional cases in Hong Kong were confirmed to be infected with influenza A (H5N1) virus and in six cases, death resulted from the infection.

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2. Investigation associated with the first cases of influenza A H5N1 infection

In view of the significance of this virus, the Department of Health of the Hong Kong Government established a Special Working Group on avian influenza virus (H5N1) to investigate this incidence. The main objectives of the initial investigation were:

(1) to rule out the possibility that the virus isolated was a laboratory contaminant;
(2) to identify the source of the virus and its association with local poultry;
(3) to determine the mode and extent of transmission.

The possibility that the virus was a contaminant was ruled out after extensive review on the processes of specimen collection, handling and transportation and the extent of communication with other research laboratory on avian influenza viruses. In addition, the presence of H5N1 infected cells in the original clinical specimen was confirmed by immunofluorescence staining using H5-specific monoclonal antibodies and reverse transcriptase polymerase chain reaction (RT-PCR). Furthermore, influenza A H5N1 virus was repeatedly isolated from the original specimen and was the only virus isolated.

Prior to this case of human infection by the H5N1 virus, an outbreak of avian influenza in chicken farms in Hong Kong was reported in March 1997 by the Department of Agriculture and Fishery in Hong Kong and an influenza A virus was isolated. High mortality in infected chickens was noted and over 4000 chickens had died. The virus was subsequently identified as influenza A (H5N1) in Professor Ken Shortridge's laboratory. In order to identify the source of the human H5N1 virus, interviews were carried out with the child's family, teachers and students of the school and healthcare workers taking care of the child to assess the linkage of the index case to the exposure to poultry. Although the infected child was in contact with live poultry in the school, there were no evidences linking the child with the outbreak of H5N1 virus in chicken farms. However, genetic sequencing of the H5N1 viruses isolated from the child and from affected chickens revealed that they were virtually identical viruses.

Table 1
Serological survey of H5N1-exposed and non-exposed individuals[a]

Group	Number tested	Number positive
Exposed group		
Case contact	365	3 (0.8)
Laboratory workers	71	1 (1.4)
Persons exposed to poultry	40	1 (2.5)
Total exposed group	476	5 (1.1)
Non-exposed controls		
Hepatitis B vaccination program	199	0 (0)
Blood donors	200	0 (0)
Total non-exposed control	399	0 (0)

[a] Values in parenthesis are in percentage.

In order to determine the mode and extent of infection of H5N1 virus in human, serological prevalence survey was conducted in populations who were exposed (contact with the index case or poultry) and non-exposed controls. The exposed group consisted of persons in contact with the index case or poultry and the non-exposed controls were selected from blood donors and a cohort of children in a hepatitis B virus vaccination programme. Assays for anti-H5N1 antibodies were performed by the Influenza Branch of the Center for Disease Control and Prevention, USA and the Government Virus Unit, Hong Kong using micro-neutralization assays and Western-blot assays based on highly purified H5 antigen expressed in baculovirus system. Results of the survey are shown in Table 1. All persons in the non-exposed

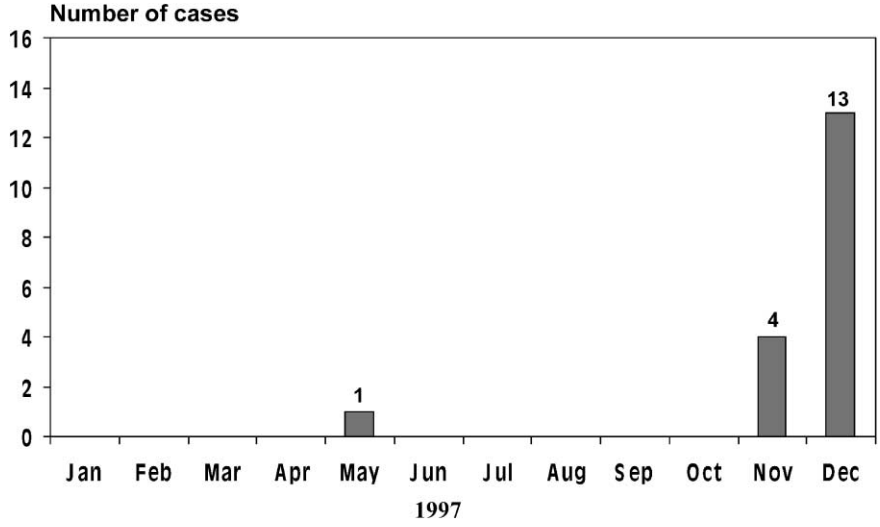

Fig. 1. Outbreak of influenza A (H5N1) in Hong Kong in 1997.

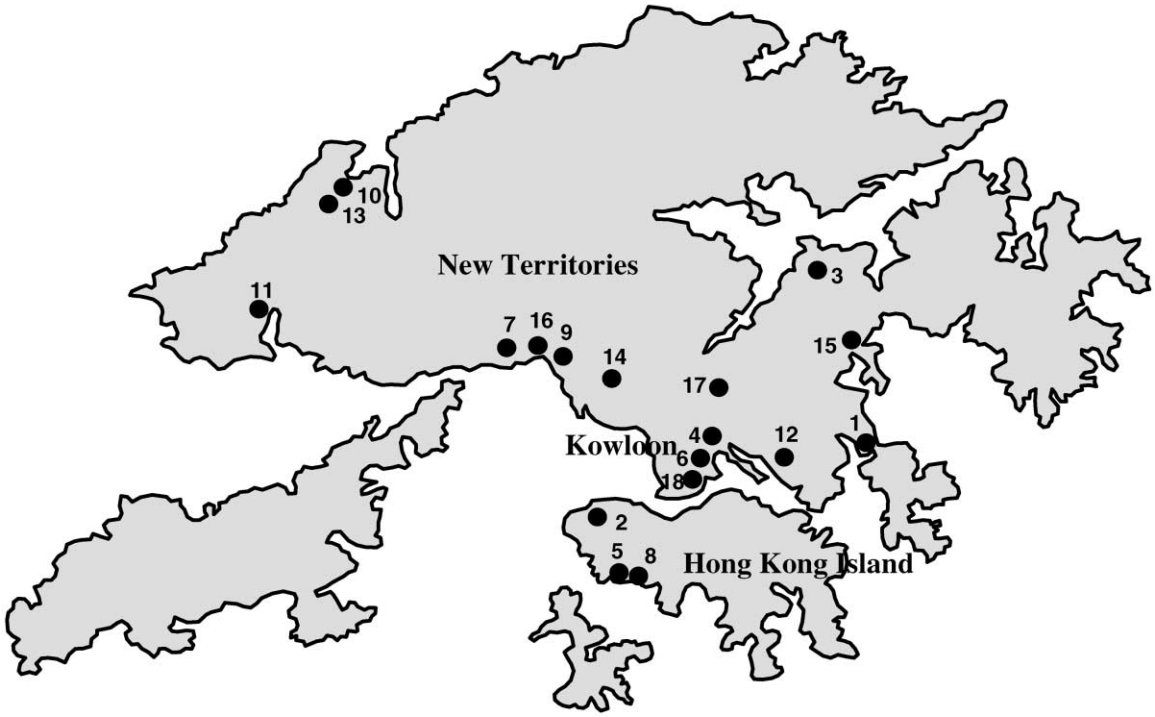

Fig. 2. Geographical distribution of influenza A (H5N1) infected cases in Hong Kong. Numbers on top of the dot designated the chronological order of appearance of the cases.

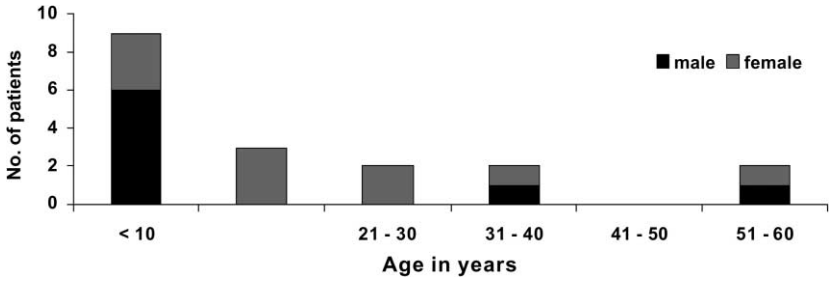

Fig. 3. Age and sex distribution of patients infected with influenza A (H5N1) virus.

group were negative for anti-H5N1 while five of the 476 persons in the exposed group were positive indicating that close contact with the index case and exposure to poultry were at risk of being infected. These results also raised the possibilities that influenza A (H5N1) virus may transmit from human to human, in addition to from birds to humans.

2.1. Outbreak of influenza A (H5N1) in Hong Kong in 1997

After the first case of H5N1 infection in May in Hong Kong, a second case of H5N1 infection was confirmed in November 1997. This was followed rapidly by a cluster of 16 more cases in the months of November and December as shown in Fig. 1. The occurrence of these cases were distributed over different part of Hong Kong with high population density as demonstrated in Fig. 2.

Of the 18 cases, 10 were females. Nine out of the 18 cases were children of 10 years of age or younger, as shown in Fig. 3. Twelve cases had a history of exposure to poultry. The index child had contact with chickens and ducklings at school. Three cases purchased poultry from the market before onset of illness and in another two cases, live chicken outlet was present nearby their home in which H5N1 virus was isolated during investigation of these two cases. Five other cases had regular visits to the markets and also live chicken outlets and one worked in the market.

3. Clinical presentation and laboratory diagnosis of influenza A (H5N1) infection

The spectrum of clinical outcome varies from asymptomatic/mild upper respiratory illness to severe pneumonia

and death. Of the 18 cases of H5N1 infection, seven were considered to have mild disease with presentation of fever, sore throat, cough, conjunctivitis and mild gastrointestinal symptoms. Eleven were considered to have severe disease with additional symptoms of pneumonia, elevated liver enzyme, renal failure and pancytopenia. The clinical features of the seven mild and 11 severe H5N1 infected cases are shown in Table 2.

The clinical progression can be divided into three phases. Phase 1 illness presented as asymptomatic or with mild upper respiratory tract infection and fever. Phase 2 is characterized with additional symptoms of severe pneumonia, haemoatological, liver and renal abnormalities and phase 3 is presented with advanced illness of acute respiratory distress syndrome and multiple organ dysfunction syndrome and eventually death. The case fatality ratio was particularly high for those over 12 years of age (five out of nine deaths) as compared to those with age 12 years or under (one out of nine deaths).

Laboratory diagnosis had been particularly difficult in the beginning of the outbreak. The virus was readily isolated in MDCK cells but subtyping of isolates was difficult due to the lack of specific antisera. With the provision of monoclonal antibodies against the H5 protein by Dr. R.G. Webster of St. Jude Children's Research Hospital, USA and the development of RT-PCR for the detection of *H5* gene locally at the Department of Microbiology, University of Hong Kong, a tentative diagnostic algorithm for the rapid diagnosis of H5N1 infection was used. (Fig. 4). The diagnostic algorithm was employed in all hospitals under the Hong Kong Hospital Authority and rapid diagnosis for H5N1 was provided by regional centres in the Hong Kong Island, Kowloon and the New Territories regions (Fig. 2).

Table 2
Clinical features of mild and severe influenza A (H5N1) virus infection

	Mild ($n = 7$)	Severe ($n = 11$)
Mean age (years)	2.8	26.3
M/F ratio	5:2	3:8
Number of patients with underlying illness	2	4
Mean body temperature at admission	38.9	39.2
Number of patients with upper respiratory track infection symptoms	7	10
Number of patients with lower respiratory track infection signs	0	11
Number of patients with gastrointestinal track symptoms	2	8
Mean white blood cell count at admission ($\times 10^9$/l)	11.2	3.9
Number of patients with lymphopenia	0	11
Number of patients with pancytopenia	0	2
Number of patients with elevated liver enzyme (ALT)	1	10
Number of patients with abnormal renal function	0	4

In the diagnostic algorithm for H5N1 infection, nasopharyngeal aspirates of patients presenting symptoms of

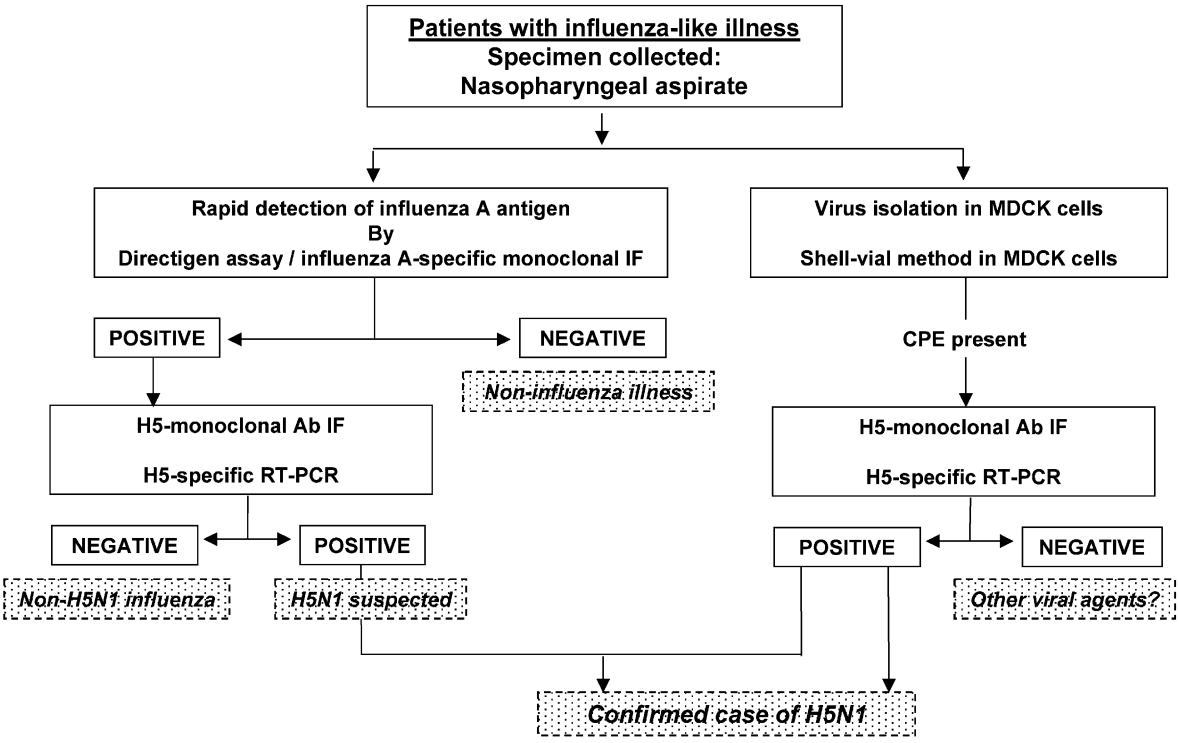

Fig. 4. Diagnostic algorithm for the rapid diagnosis of H5N1 infection.

influenza-like illnesses were first tested for influenza A antigen by the Directigen assay or monoclonal-based influenza A specific immunofluorescence. If influenza A antigen was detected, further direct testing of the specimen for H5 antigen by H5-specific monoclonals immunofluorescence staining and/or H5-specific RT-PCR were done. Patients were classified as suspected H5N1 infection if H5-specific protein or H5-specific gene is detected. Virus isolation and shell-vial cultures for H5N1 using MDCK cells were also performed for all patients with influenza-like illnesses. Viruses isolated were identified as H5 by monoclonal immunofluorescence staining and/or RT-PCR. Only patients with positive H5N1 virus isolation were classified as confirmed H5N1 infected cases. Such algorithm provided a rapid initial result for patient management and infection control in all hospitals within 24 h.

3.1. Control measures for the spread of influenza A (H5N1) in Hong Kong

Control measures for the spread of H5N1 in Hong Kong included enhanced surveillance on human infection and the extent of spread in the domestic poultry industry. Import control of poultry from China (the main source of live poultry for Hong Kong) was instituted by the government of Hong Kong and guidelines were issued to all operators of food premises and poultry outlets for the improvement of hygienic conditions in poultry farms and markets. Supervised cleaning of poultry farms, wholesale and retail outlets for poultry was enforced.

In the later part of December 1997, there had been a continuous appearance of new human cases of H5N1 infection and H5N1 virus was isolated from the wholesale market for poultry continuously despite the introduction of control measures. In addition, there was no effective treatment for H5N1 infection and vaccine for H5N1 viruses was not available for disease prevention.

The government of Hong Kong decided on 28 December, to cull all chickens in Hong Kong and 1.5 million of birds were killed from 29 to 31 December 1997. Such measures had been successful in controlling new human cases of H5N1 infection and no further cases were detected since. In addition, a series of control measures were introduced to ensure H5N1-free poultry in Hong Kong. These included the segregation of water fowl and chickens in the wholesale and retail level and the central slaughtering of water fowl since ducks and other aquatic birds were suspected to be carriers of the H5N1 virus. Quarantine and testing of import chickens at the borders were introduced. Only lots of chickens certified to be H5N1-free (H5 antibody negative) were permitted to be imported.

3.2. Epidemiological factors leading to the outbreak of avian influenza A (H5N1) in Hong Kong

Many factors may have contributed to the sudden outbreak of H5N1 virus in Hong Kong. These may include:

- the traditional Chinese requirement of purchasing live chicken for various cultural activities and the believe in the freshness of recently slaughtered birds;
- the crowded conditions of Hong Kong and the close proximity of live birds markets to residential complexes;
- the unhygienic way of slaughter and crowded conditions where live bird were kept in these markets;
- the close proximity of various poultry in traditional markets and wholesale markets where different species of birds were kept.

Under such conditions, the virus can be easily spread from one species of bird to another and eventually to human. Characteristics of the H5N1 virus may have contributed to the spread of the virus and the severe disease associated with infection. The virus is highly virulent which causes high mortality in infected chickens and caused the death of six out of 18 infected patients. It possess a mutated haemagglutinin which is highly susceptible to proteolytic cleavage. Such property had been associated with high virulence and may have contributed to its ability to cross the species barrier from avian species to humans.

Acknowledgements

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