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Novel Avian Flu A (H7N9): Clinical and Epidemiological Aspects, and Management

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Abstract

Avian influenza A (H7N9) virus, the latest avian influenza virus strain was once considered a relatively rare cause of infection and low pathogenic. This novel virus spreading among three cases was firstly reported on 31 March 2013 in eastern China. By 31 March 2013, the number of laboratory-confirmed influenza H7N9 virus infections reached 132, with 37 deaths. There are age and gender differences between H7N9 virus-infected and H5N1 virus-infected patients. Currently, the reservoir or source of this novel virus is unknown but is most likely to be live-bird markets in eastern China. Low pathogenicity in poultry and birds and no evidence of human-to-human transmission have been noted. The incubation period of this novel virus ranges 3 to 8 days, thus the most appropriate time for contacts observation and treatment is 10 full days. The best diagnostic method for confirmation of H7N9 virus infections is real-time reverse-transcriptase polymerase chain reaction. Lymphopenia and thrombocytopenia can be predictors of acute respiratory distress syndrome and death. Approximately, 20% of cases without appropriate treatment with antivirals are dead with respiratory failure and multiple organ failure. Fortunately, Oseltamivir and Zanamivir are still susceptible to this novel virus. Currently, no specific vaccines against H7N9 viruses are available. China scenario and its model against spreading of this virus can be an effective model for other countries for protection of this virus spreading.

Keywords: H7N9; Avian Flu; Clinical; Epidemiological; Management

Introduction

Avian influenza A (H7N9) virus, the latest avian influenza virus strain was once considered a relatively rare cause of infection and low pathogenic [1,2]. This virus is similar to its closer cousins, H7N2, H7N3 and H7N7 and its more distant cousin H5N1 viruses which are all influenza A viruses and usually infect birds [1]. The virus has 8 single stranded ribonucleic acid (RNA) segments with 11 proteins (cap recognition RNA polymerase (basic) (PB2), endonuclease, elongation RNA polymerase subunit (basic) (PB1: Pol & PB1-F2), RNA polymerase subunit (acidic) (PA), hemagglutinin (HA), Nucleoprotein RNA binding RNA synthesis (NP), neuraminidase (NA), matrix protein 1 (M1), ion channel (M2), NS1 and NEP encoding [1]. A schematic picture of an influenza virus is shown in Figure 1 [1].

Epidemiology and Outbreak

On 31 March 2013, the Government of China in accordance with the International Health Regulations (2005) (IHR) reported three cases of the Mainland - Health - Authorities laboratory - confirmed human infection with a novel influenza A (H7N9) in Shanghai and the province of Anhui [2,3] whereas no bird influenza viruses were identified in dead pig specimens from a river that provided drinking water to residents in Shanghai where two people died in the first human infections with a novel avian influenza strain [4]. Two cases with influenza A (H7N9) infection were detected in the residents of the city of Shanghai and another case in a resident of Anhui province [2]. The first case was an 87-year-old male patient from the city of Shanghai who reported the onset of influenza-like symptoms on 19 February 2013 and he was dead in Shanghai after 14 days of falling ill [4] whereas the second and third cases had illness on 27 February and 15 March 2013, respectively [2]. The cause of death in the first case appeared to be multiple organ failure [4]. The second case from Shanghai was the 27-year-old male butcher who could not be ruled out infection with virus in poultry meat stalls while earlier the Shanghai Huangpu River found a large number of dead pigs floating and finally, the Mainland Health Authorities found no avian flu virus in these dead pigs while in the past, this avian flu virus was found only in poultry [4]. The second case was dead in Shanghai 11 days after falling ill [4]. Two Shanghai patients with market slaughter trafficking animal activities contacted other Shanghai patients without a history of contact with animals but two sons of the first case had also pneumonia admissions [4]. Cause of illness in these two sons have not been yet known, but naturally, if three individuals in one family acquire severe pneumonia in a short period of time, it raises a lot of concern [4]. The third case with critical condition from Anhui was 35-year-old woman who had history of exposure to poultry before the onset of symptoms [4]. She was still alive 23 days after falling ill (31 March 2013) [4]. This virus showed no signs of being highly contagious among humans according to the cases of close contacts [4]. So far, three cases diagnosed with the specific source of infection are still unclear [4]. As of 1 May 2013, there had been 128 laboratory-confirmed cases of influenza A (H7N9) virus distributed over 39 prefectures or districts in 10 provinces or municipalities including Beijing (1 case, 0 death), Shanghai (33 cases, 13 deaths), Jiangsu (27 cases, 6 deaths), Zhejiang (46 cases, 6 deaths), Anhui (4 cases, 1 death), Fujian (3 cases, 0 death), Jiangxi (5 cases, 0 death), Shandong (2 cases, 0 death), Henan (4 cases, 0 death) and Hunan (2 cases, 0 death) [5]. Only three cases among children were laboratory-confirmed [5]. Of the 128 laboratory-confirmed cases, 26 (20%) died and 26 (20%) were known to have recovered [5].

To date, no epidemiological association between the cases has

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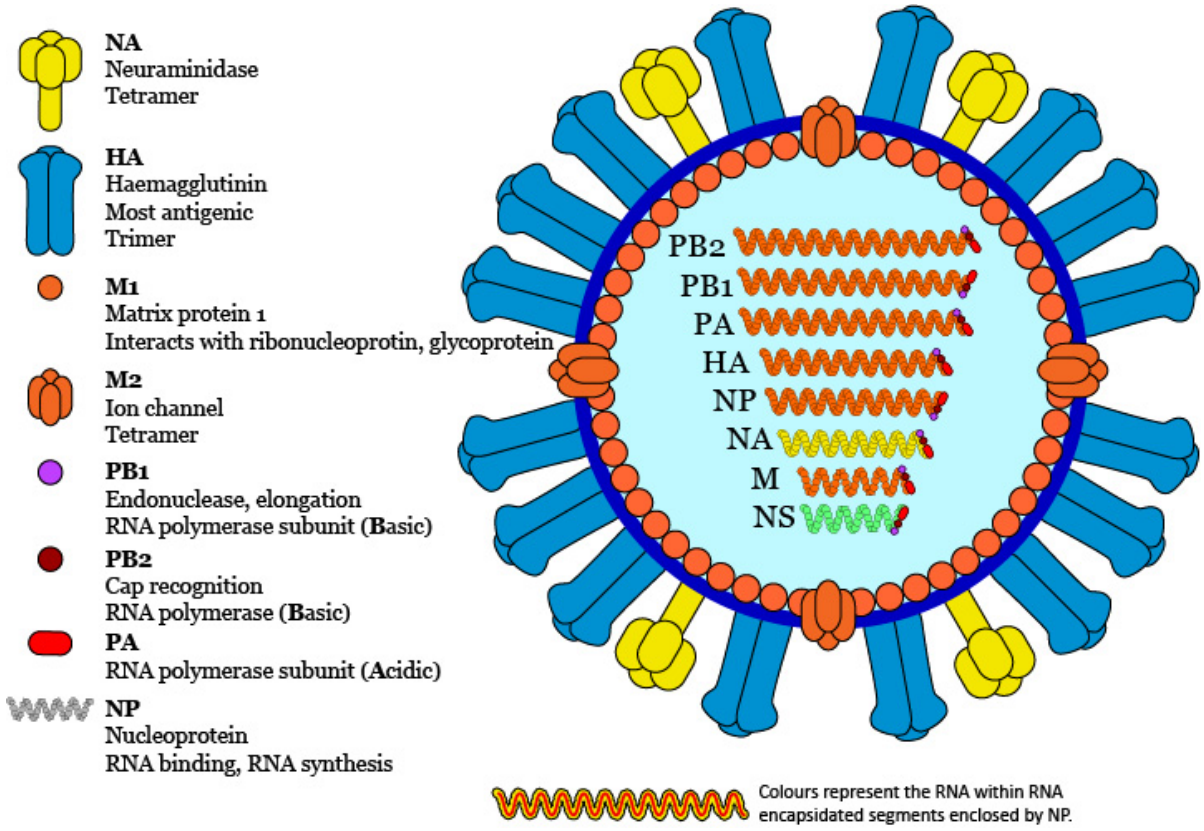

Figure 1: A schematic picture of an influenza virus [1].

been found [4]. Approximately 2 months after the initial report (by 31March 2013), the number of laboratory-confirmed influenza H7N9 virus infections reached 132, with 37 deaths, originating from the above locations and seven additional provinces, Fujian, Jiangxi, Jiangsu, Henan, Hunan, Shandong, Zhejiang, and the municipality of Beijing, in addition to one case with a history of recent travel from Jiangsu reported by Taipei, Centers for Disease Control (CDC) [2]. All locations were in eastern and northern China. On 31 March 2013, the Chinese Center for Disease control and Prevention (China CDC) posted full genome sequences of viruses isolated from the first three cases in a publicly accessible database [3]. Underlying chronic medical conditions were reported in most cases (54 of 128 laboratory-confirmed cases, 76%) [2,5] who initially developed an influenza-liked illness (ILI) that subsequently progressed to respiratory distress syndrome [2] or severe pneumonia [3] resulting in hospitalization. The case fatality approximately reached 25%, which was a provisional value because many cases remained hospitalization as of 8 May 2013 while the number of mild cases remained unknown [2].Six cases were detected through ILI surveillance whereas two of them with mild symptoms did not require hospitalization [2]. The majority of the patients were 61-62 years of age [2,3,5] with 2 - 2.4:1 male to female ratio [2,5]. In contrast, previous infections with subtype H7 avian influenza viruses had generally been mild and associated with conjunctivitis [2]. Off 77 cases nationally reported for which data were available, 18 (23%) reported no detected contact with poultry whereas 56 (72%) reported some recent contacts with live poultry and live poultry markets [3]. Almost all cases had been sporadic but three family clusters had been detected [3]. Of more than 3,000 close contacts, 19 developed respiratory symptoms [3]. None of these symptomatic cases tested positive for H7N9 virus infections by reverse-transcriptase polymerase chain reaction (RT-PCR) while results of serology testing were pending [3]. In Shanghai, two family clusters were detected with two laboratory-confirmed cases and one suspected cases while one of these individuals recovered but the other two cases died in February 2013 from respiratory failure [5]. The second probable family cluster was husband and wife from Shanghai with laboratory-confirmation [5]. A third family cluster was identified in Jiangsu with one laboratory-confirmed case and one suspected case [5]. Both cases were critically medical-condition hospitalized [5]. In the first two clusters, it was not possible to determine whether those infected were exposed to a common source or there had been limited person-to-person transmission [5]. Surveillance for ILI among individuals in close contact with laboratory-confirmed influenza H7N9 cases indicated that infected persons were not a likely source of infection [2,3]. These preliminary studies suggested that despite numerous cases of influenza H7N9 virus infection associated with poultry exposure, there was no sufficient evidence of sustained onwards virus transmission to other persons [2,3].

No new cases have been found since a "zero report" system was implemented by the City of Chuzhou and the First People's Hospital of Chuzhou City was determined for patient admission [4]. The China's human influenza surveillance system is not reporting an overall increase in influenza virus detection or atypical pneumonia cases in the most recent reporting period [5]. Although there have been increased reports of ILI in the sentinel systems in the first affected areas, analyses of the underlying viruses have found hardly any A (H7N9) virus but a number of seasonal influenza viruses [5]. This increase in ILI reports is therefore probably explained by increased care seeking and testing encouraged by health authorities [5]. Hence, the available epidemiological data are not compatible with efficient human-to-

human transmission of influenza A (H7N9) virus [5]. In consideration of comparisons between influenza A (H7N9) and A (H5N1) virus infection in humans, there are most notably and considerably greater number of human A (H7N9) virus-infected cases presumed to come from animal or the environment exposure more than weeks (over 100 laboratory-confirmed cases), compared with only 43 human cases of influenza A(H5N1) virus infection in the mainland of China between 2003 and 2013 [5].

Prior exposure to influenza A (H7N9) viruses has been cited as a possible mechanism to explain the age effect but this does not readily explain the gender differences which might also reflect differences in exposure, care seeking or visiting markets whereas there is an unusual and unexplained age and gender distribution in China of the cases with influenza A (H7N9) virus infection [5]. Cases of influenza A (H7N9) virus infection are considerably older than for A (H5N1) virus infected cases and male influenza A (H7N9) virus-infected cases are more than two folds as numerous as female cases for influenza A (H7N9) virus whereas there were roughly equal numbers for influenza A (H5N1) virus-infected cases [5]. The human influenza A (H7N9) virus transmission is probably facilitated by the fact that many people in China still buy live poultry for domestic consumption [5]. Nevertheless, other possible animal reservoirs remain to be investigated [5]. There is yet no information available from analytical studies of risk factors for H7N9 virus infection such as behavioral or case-control studies [5]. However, human influenza A (H5N1) virus infection appears to be related to exposure to contaminated environments or live poultry because:

1) The virus has been detected in poultry in live-bird markets,

2) Most human cases (approximately three out of four cases) reported a history of exposure to animals, mostly chickens,

3) The number of human cases appears to have decreased after closure of live-animal markets, and

4) The virus in humans is genetically similar to that found in the environment (live-bird markets) and in animals [6]. A total of 61.3% of the cases had one or more coexisting medical conditions [7]. Underlying chronic medical conditions were demonstrated in most cases [2]. Chronic obstructive pulmonary disease, diabetes, hypertension, and coronary heart disease were the most coexisting conditions [7]. Two cases were pregnant, one in the first trimester and the other in the second trimester [7]. Currently, Chinese close contacts under medical observation for 10 full days [4,5], close contacts track retrospective follow-up, and the source of infection is still ongoing [4].

Clinical Manifestations

The current presumed incubation period is average 5.8 days (3-8 days) [1,8] but some investigators recently reported that it was 5 days (interquartile range, 2 to 8) [7]. The median time from illness onset to hospital admission is approximately 4.5 days with a high proportion of patients with intensive care admission [2]. A small number of clinically mild H7N9 virus infections with uncomplicated influenza (febrile upper respiratory tract illness) had been detected in children and adults [2]. All first three cases presented with respiratory tract infection with progression to severe pneumonia and breathing difficulties [4]. Table 1 and 2 demonstrates clinical findings upon presentation and its complications; respectively. Multiple organ failure was the usual cause of death (20%) [2,5].

Most human cases had resulted in clinically severe illness [6]. The median time from the onset of illness to shock was 8 days [7]. The median time from onset of illness to ARDS was 7 days [7]. The median time from illness onset to death is approximately 11 days [2]. The leukocyte counts was normal or slightly decreased [2,7] with leukopenia [2,7,8] and lymphopenia (88.3%) [2,7,8]. Moderate thrombocytopenia was noted in some cases (39.6%) [2,7]. There was elevation of serum lactate dehydrogenase, serum aspartate aminotransferase and serum creatinine kinase in the majority of the cases [7,8]. Substantially increased serum chemokines or cytokines concentrations and disseminated intravascular coagulation with disease progression were noted [8]. The sputum examination results were more likely to be positive for the H7N9 virus than were the throat swab specimens [8]. On hospital admission, 97.3% of cases had radiological findings that were consistent with pneumonia that most commonly presented with bilateral ground-glass opacities and consolidation [7].

Laboratory Diagnosis

Three laboratory diagnostic methods were used: H7N9 serological testing (modified hemagglutination-inhibition (HI) assays), viral isolation, and real-time RT-PCR [7]. Between 2 and 13 April 2013, real-time PCR primers and probes based on the sequences of the first three H7N9 isolates were distributed within 48 hours by the China CDC to more than 400 influenza surveillance and other diagnostic network laboratories throughout China [2,3] including reverse agglutination serological testing [1]. The World Health Organization (WHO) Collaborating Centers on influenza including the United States Centers for Disease Control and Prevention (US CDC) and the Japan National Institute for Infectious Diseases had also developed and shared H7N9 specific PCR reagents [2]. The WHO Global Influenza Surveillance and Response System (GISRS) and partner laboratories have developed microneutralization laboratory and HI protocols to identify specific H7N9 virus antibodies in human sera [2]. Protocols, primers and probe sequences of the real-time RT-PCR which included a housekeeping/endogenous gene control were released on the WHO's website [1,3]. All PCR-confirmed human cases had been diagnosed using these

Sign and Symptom	Reference
High fever	[1,2,4,7]
Weakness	[1]
Influenza-productive cough	[1,2,4,7]
Conjunctivitis	[5]
Chills	[1]
Influenza-liked illness	[5]
Diarrhea or vomiting	[7]
Shortness of breath	[1,2,4]
Dyspnea	[1,2,5,7]

Table 1: Clinical signs and symptoms of patients infected with avian influenza A (H7N9) virus.

Complication	Reference
Viral or severe pneumonia	[1,2,4,5,7]
Bacterial or fungal ventilator-associated pneumonia	[2]
Hypoxia or refractory hypoxemia	[2]
Acute Respiratory Distress Syndrome	[2,5,7]
Septic shock	[2,7]
Encephalopathy	[2]
Rhabdomyolysis	[2,7]
Acute renal dysfunction	[2,7,8]
Respiratory failure	[2,5]
Multiple organ failure	[2,5]

Table 2: Complications in patients infected with avian influenza A (H7N9)virus.

reagents [3]. In Europe, all European Member States were expected to urgently send un-subtypeable A viruses and subtyped A (H7) tested by generic RT-PCR assays for influenza A virus which could likely identify novel viruses to the WHO Collaborating Center in London for further virus characterization [5].

To assist the European laboratories in ensuring and verifying their diagnostic capability to identify H7N9 viruses, the European CDC jointly with the WHO Regional Office for Europe released a technical briefing note on Diagnostic preparedness in Europe for detection of H7N9 viruses as the following:

1) A list of laboratory preparedness considerations to ensure European-wide diagnostic capability,

2) An update on current methods used for molecular identification of human infection with H7N9 viruses by RT-PCR assays,

3) A table of H7 HA assay validation criteria, and

4) Information on positive controls for RT-PCR assays [5].

Preliminary human serological assays in China had been undertaken using antigens from A/Anhui/1/2013 with early data demonstrating that sera from children, adults and the elderly lacked antibodies to H7N9 virus before and after immunization with 2012-2013 seasonal influenza vaccine [3]. Assays of avian serum specimens to date have used A (H7N2) antigens and antisera [3]. Availability of specific post-infection antisera raised against H7N9 viruses in ferrets or chickens presently waits for full development of H7 N9-specific serological assays for human and animal use [3]. Clinical samples from the first three cases of H7N9 virus infections were initially reported as positive testing results for influenza A viral RNA but un-subtypeable by real-time RT-PCR testing which routinely used by the public health laboratories [2,5]. These tests were designed to identify only whether the samples contains influenza type A or B viral RNA from a respiratory specimen and for type A positive specimens to detect the HA gene as subtype H5 from avian influenza A (H5N1), H1 or H3 from A (H1N1) or A (H3N2) seasonal influenza viruses, respectively [2]. Therefore, the real-time RT-PCR testing results designed for presently circulating seasonal viruses or A (H5N1) were reported as influenza A viruses of unknown subtype by the municipal and provincial public health authorities [2]. Further real-time RT-PCR tests and sequence analysis of these clinical samples at the China National Influenza Center in Beijing demonstrated that the HA belonged to the H7 subtype and the neuraminidase (NA) belonged to the N9subtype [2] whereas real-time RT-PCR testing for other respiratory pathogenic microorganisms revealed negative results [2].

A recent study among 111 cases of influenza A (H7N9) virus infection demonstrated that other viruses including seasonal influenza viruses (H1, H3, or B), H5N1, severe acute respiratory syndrome coronavirus (SARS-Cov), and human coronavirus-Erasmus Medical Center (HCoV-EMC) were also identified by real-time RT-PCR methods in most cases [7]. Currently, real-time RT-PCR assay with primers and probes designed to identify the Eurasian H7 hemagglutinin is the method of choice to analyze respiratory samples for diagnosis of H7N9 virus infection [2]. Cultured Madin-Darby canine kidney cells supplemented with trypsin, and embryonated chicken eggs with supporting the growth of the H7N9virus in clinical specimens are the other two routine-laboratory methods which can measure the quantity of virus in culture media by agglutination of erythrocytes derived from turkey guinea pig, chicken, or horse [2]. The WHO GISRS laboratories recommend using Turkey red blood cells in measurement of quantity of the H7N9 virus in culture media [2].

Taiwan had previously developed an in-house RT-rtPCR newly designed RT-rtPCR assays based on an alignment of 3 Chinese H7N9 sequences, targeting HA (two assays) and NA and tested using A/Mallard/Sweden/91/2002 (H7N9) RNA [1]. A mutated (traceable to discriminate laboratory contamination) cloned positive control was produced publicly available to investigators [1]. Taiwan's CDC recommended hospital collect a lower respiratory specimen such as sputum [1]. RT-rtPCR testing for a sputum specimen showed positive result among two negative testing results on throat swabs [1]. The US CDC has presently released its RT-rtPCR and protocol with support from the US Food and Drug Administration (FDA) [1]. This assay also contains an RNaseP endogenous control as does the WHO protocol [1]. There is evidence of assay variation, supporting underlining the need for independently designed, compared and validated molecular assays and sharing of the testing results [1].

Genetic Characterization of the H7N9 Viruses

A nucleotide sequence alignment comparison of each the eight genes of the first three viruses isolated from humans in China was initially carried out by the WHO Collaborating Centers for Reference and Research on Influenza in Beijing and additional genetic analyses were out by the WHO Collaborating Centers for Reference and Research on Influenza in London, Atlanta, Tokyo and Melbourne [5]. The three viruses were very similar to each other and shared greatest identity with genes of avian influenza viruses that recently circulated in China and their complete genomic coding sequences were deposited into the Global Initiative on Sharing Avian Influenza Data (GISAID) database on 31 March 2013 [2,5] and also in the INSDC databases [5]. In Europe, a first summary was published on the WHO Regional Office for Europe website and further publications had come from Beijing and Tokyo and a summary from Atlanta [5]. Analyses of the first three virus isolates demonstrated that the viruses were reassortants comprising H7 HA, N9 NA and the six internal genes of H9N2 influenza A viruses recently isolated from poultry in China [3,5]. The genotype of H7N9 influenza viruses isolated from humans might have originated in China by reassortment of poultry A (H9N2) viruses with duck viruses carrying H7 and N9 genes [2,5]. Additional viruses with reassortant genomes are likely to be identified as more sequence data become available [2]. While nucleotide sequences of all three viruses were closely related, A/Shanghai/2/2013 and A/Anhui/1/2013 were nearly identical to each other across all 8 gene segments more than to A/Shanghai/1/2013 which was distinctive at multiple sites [2,3]. This gene constellation had not been previously detected among viruses obtained from birds, humans or any other species including those reported in birds in Europe [3,5] and were most closely related to a previously undetected avian influenza virus with genes derived from numerous potential parental strains [2]. The first H7N9 full genomes (all 8 segments) which were submitted to be collected in GISAID databases are shown in Table 3 [1].

Comparisons to other influenza A virus sequences in public databases demonstrated that the most closely related viruses were recent low-pathogenic Eurasian H7N9 viruses (for example, A/wild bird/Korea/A14/2011 (H7N9)), H7N3 viruses (for example, A/duck/Zhejiang/12/2011 (H7N3)) and H9N2 viruses (for example, A/brambling/Beijing/16/2012 (H9N2)) [3]. The HA genes had highest levels of sequence identity (95%) with H7N3viruses recently identified in ducks at live-bird markets in eastern China [2] whereas the NA genes were highly similar (96% identity) to N9NA genes from viruses recently circulating in domestic ducks in China and Korea but featured a distinctive 15 nucleotide deletion (amino acid 69-73) beginning at position 215 [2]. The rest of six viral genes (PA, PB1, PB2, NP, M

H7N9 viral isolates	Date of viral isolates submitted to GISAID
A/Pigeon/Shanghai/S1069/2013, Harbin Veterinary Research Institute	7 April 2013
A/Environment/Shanghai/S1088/2013, Harbin Veterinary Research Institute	7 April 2013
A/Chicken/Shanghai/S1053/2013, Harbin Veterinary Research Institute	7 April 2013
A/Environment/Nanjing/2913/2013, environment, EpiFluDB	14 April 2013
A/Nanjing/1/2013, human EpiFluDB	14 April 2013
A/Hangzhou/1/2013, 38-year-old man, Hangzhou Center for Disease Control and Prevention	19 April 2013
A/Environment/Hangzhou/34/2013, feces, Hangzhou Center for Disease Control and Prevention	22 April 2013
A/Hangzhou/2/2013, 67-year-old man, Institute of Microbiology, Beichen West Road, Chaoyang District, Beijing 100101	22 April 2013
A/Hangzhou/3/2013, 79-year-old man, Hangzhou Center for Disease Control and Prevention	22 April 2013
A/Anhui/1/2013, 35-year-old woman, WHO Chinese National Influenza Center	27 April 2013
A/Shanghai/1/2013, 87-year-old man, WHO Chinese National Influenza Center	27 April 2013
A/Shanghai/2/2013, 27-year-old man, WHO Chinese National Influenza Center	27 April 2013
A/Zhejiang/1/2013, 39-year-old man, Zhejiang Provincial Center for Disease Control and Prevention	28 April 2013
A/Zhejiang/2/2013, 64-year-old man, Zhejiang Provincial Center for Disease Control and Prevention	28 April 2013
A/Zhejiang/01/2013, 39-year-old man, WHO Chinese National Influenza Center Virology Institute, Chinese CDC	27 April 2013
A/Taiwan/1/2013, 53-year-old man, National Influenza Center, Centers for Disease Control, Taiwan	9 May 2013

Table 3: H7N9 viral isolates in 2013 China's outbreak [1].

and NS) had greatest identity (99%) with A (H9N2) poultry viruses which have been circulated in China since 1994 [2]. The HA genes of A/Hangzhou/1/2013 and A/Anhui/1/2013 clustered with A/chicken/Shanghai/S1053/2013 and A/pigeon/Shanghai/S1069/2013 as same as A/Shanghai/2/2013 human isolates while A/Shanghai/1/2013 was more divergent [2]. The HA genes from this outbreak clustered with A (H7N3) viruses from ducks recently sampled in this region, such as A/duck/Zhejiang/12/2011 (H7N3) and their genetic distances were consistent with limited unsampled evolution whereas the NA genes descend from an ancestor of duck viruses recently identified in this region, such as A/wild bird/Korea/A9/2011 (H7N9) [2]. The segment encoding HA belonged to the Eurasia A (H7) avian influenza virus lineage whereas the segment for NA was most identity to avian H11N9 and H7N9 viruses [5].

All internal genes of H7N9 viruses seem to belong to H9N2 viruses [4]. Presently, some H9N2 viruses are also reassortants and a number of these viruses have NS and PA gene segments from the influenza H5N1 viruses [4]. Analyses of the 11 virus protein sequences deduced from gene sequences of H7N9 viruses provided critical insight into their biological and evolution properties [2]. The HA protein of the viruses are characterized by the presence of a single basic amino acid at the HA0 cleavage site that yieldsHA1 and HA2 [2]. No amino acid deletions or insertions were detected in the HA sequence [2]. The presence or absence of multiple basic amino acid or other sequence insertions at the cleavage site of the HA0 which is one of the criteria used to determine the virulence potential of influenza viruses for chickens and other avian species did not appeared these 11 virus protein sequences supported its classification as "low - pathogenic" for chickens [2] and birds [6], but did not guarantee the capacity of these viruses to cause severe and fatal human infections [2]. The substitution of Q226L in the HA gene which has been associated with reduced binding to avian-like receptors and with sialic acids linked to galactose by α-2,3 linkages (found in the human lower respiratory tract) occurred in almost all the human and non-human H7N9 isolates [5]. These *in vitro* analyses concluded that all three viruses bound both α-2,3- and α-2,6 –linked sialic acids and indicated an ability to bind both avian and mammalian cells [3]. This substitution is also associated with enhanced ability to bind to mammalian-like receptors bearing sialic acids linked to galactose by α-2,6 linkages, which are found in the upper respiratory tracts of humans and other mammals [3,5,6].

A recent study demonstrated that there were Gln226Leu and Gly186Val substitutions in human virus H7which are associated with increased affinity forα-2,6 –linked sialic acid receptors [8] and the PB2 Asp701Asn mutation which is associated with mammalian adaptation [5,8]. Most but not all human isolates had 226L in the HA gene which is known to conduct enhanced binding to the α-2,6-linked sialic acid receptors found in human upper respiratory tract [3].

With the exception of A/Shanghai/1/2013 which demonstrated a Lys to Arg amino acid substitution at position 289 (292 in N2 numbering) which was predicted to affect susceptibility to NA inhibitor agents, the NA active site residues were conserved in all H7N9 outbreak viruses [2]. The PB2 proteins from some H7N9 viruses isolated from humans demonstrated substitutions in the genes by mutations at positions 627 (Glu to Lys in the human isolated from Anhui, Hangzhou and Shanghai) or 701 (Asp to Asn in A/Zhejiang/DTID-ZJU01/2013) and these are known to enhance the replication of avian influenza viruses at temperatures similar to that of mammals and possibly humans as well [2,5]. In comparison to the PB2s from H7N9 viruses which isolated from birds retained Glu at position 627 and Asp at 701, strongly indicating that the mutation was positively selected upon replication in humans as previously reported for zoonotic A (H7N7) and A (H5N1) infections [2]. Either E627K [3,5] from some human isolates or D701N gene in different strains have been demonstrated in PB2 gene which are both markers of mammalian adaptation [5], particularly E627K gene is associated with viral replication at the lower temperature of the mammalian respiratory tract [3]. Data analyzed by the National Avian Influenza Reference Laboratory, Habin, China indicated that all tested avian and environmental isolates had 627E in PB2 gene, all apart from A/Shanghai/1/2013 had R292 in the NA gene, and all H7N9 viruses circulated in eastern China lacked a multi-basic amino acid cleavage site in the HA gene [3]. It was remarkable that the 39 poultry or environmental isolates had the mammalian adaptation marker "PB2 627K" whereas more than half of the human isolates did [3]. Adaptation within infected humans might explain this remark; nevertheless, other possibilities cannot be excluded at the present time [3].

Unfortunately, an M2 gene marker S31N demonstrating a Ser to Asn mutation at position 31 (Ser31Asn mutation) which associated with Adamantine resistance was detected in the first three human isolates [1-3,5,8], therefore, expected that the viruses will be resistant to Amantadine and Rimantadine while these two antivirals are no longer

in use in Europe [5,6]. Fortunately, the WHO Collaborating Center in Beijing has confirmed by functional assays that influenza A (H7N9) virus is susceptible to both Oseltamivir and Zanamivir in phenotypic tests [1,3,5,6,9]. In case of A/Shanghai/1/2013, the result appeared to reflect the presence of a mixture of viruses with R or K at position 292 of the NA gene [3]. A recent report demonstrated that R292K in one virus (A/Shanghai/1/2013) was associated with markedly reduced susceptibility to Oseltamivir and modestly reduced susceptibility to Zanamivir [3]. The NP gene of the A/Shanghai/1/2013 (H7N9) virus had a clearly distinctive evolutionary history as compared to the other H7N9 viruses and likewise, A/Pigeon/Shanghai/S1069/2013 (H7N9) demonstrated a similarly divergent PB1 gene of distinctive ancestry [2]. Additional markers for adaptation to non-avian hosts or virulence were shown in the PB1-F2, M1 and NS1 genes [2]. The PA genes of A/Zhejiang/DTID-ZJU01/2013 and A/Zhejiang2/2013 were also distinctive from those of the known H7N9 viruses [2].

The reservoir for the novel virus infecting humans remains unknown but the virus had been identified in domestic birds in live markets in eastern China [5] whereas a recent study compared the sequence diversity of HA, NA and PB2 genes noted during the Dutch A (H7N7) and Italian A (H7N1) outbreaks with the initial influenza A (H7N9) virus sequences from the current outbreak in China and concluded that the genetic distance noted among the available genome sequences indicated that the immediate ancestral viruses that contributed the H7 and the N9 genes remain unknown and H7N9 viruses had substantially circulated in the animal reservoir, particularly birds in Asia for several months before their recent identification in humans and animals [2,5]. The role of the Ala to Ser substitution at position128 (137 in H3) in the HA of A/Shanghai/1/2013 is not well established [2] whereas genetic markers associated with high pathogenicity in poultry , particularly in birds have not been identified by recently demonstrating of a single Arg at the HA cleavage site consistent with low pathogenicity in poultry [3] but this finding requires further confirmation by intravenous pathogenicity index testing in chickens which is now underway [5]. These viruses are the first low-pathogenicity viruses that have caused severe human disease [2,5,6] but low or zero pathogenicity in poultry does not necessary point low human pathogenicity [2,5].

Clinical Management and Antiviral Chemotherapy

According to the neuraminidase sequencing data, testing of the A/Anhui/1/2013 virus in the neuraminidase inhibition assay pointed that this virus was susceptible to neuraminidase inhibitor (NAI) antivirals "Oseltamivir and Zanamivir" but the Arg (R) to Lys (K) substitution at the residue 292 (N2 numbering) which is likely to reduce efficacy of Oseltamivir and Zanamivir was identified initially in the A/Shanghai/1/2013 virus [2,10]. One case of 14 patients was recently reported that wild-type sequence Arg292 was observed two days after beginning of chemotherapy whereas two cases who received corticosteroid treatment developed emergence of NA Arg292Lys mutation [10]. A recent study on hospitalized patients with pneumonia indicated that systemic high-dose steroid use might result in increased risk of viral replication and shedding contributing to the emergence of antiviral resistance [2]. Nevertheless, testing of A/Shanghai/1/2013 virus in the neuraminidase inhibition assays produced discrepant results which might be ascribed to a mixture of R and K at 292 residue of the virus [2]. The R292K mutants were identified from two of the three poor responders to the NAI antiviral chemotherapy with persistently high viral load in their throat [2]. In one of these two patients, the neuraminidase had 292R on day 2 of antiviral chemotherapy and 292K on day 9 indicating selection of the resistant virus to dominate the infection [2].

NAIs were prescribed to almost all patients but only after a median of 6 days after illness onset [3]. Nevertheless, some investigators have detected the presence of an important mutation within one of the three avian influenza A (H7N9) virus strains' publicly available (GISAID) genetic sequences; one that is able to confer Oseltamivir resistance [1]. The NHFPC developed a risk-based management protocol for areas where confirmed cases were reported so that the NAIs could be prescribed earlier to the symptomatic cases, even before the confirmed results of the laboratory tests for H7N9 viruses [3]. In China, free clinical care for all H7N9-infected individuals is provided by the government [3]. Fever clinics which their infection control measures complied with the national and WHO guidelines including designated hospitals were activated in all health care facilities for screening of patients and to ensure appropriate infection prevention and control and clinical management and national guidelines on influenza H7N9 case management was also issued [3]. In Shanghai, the Municipal Public Health Clinical Center which has 500 beds and hosts high-level expertise in clinical management, has hospitalized and managed the suspected H7N9 cases and extensively accumulated experience in managing and treating patients with novel virus-associated diseases [3].

The Chinese regulatory agency, the China Food and Drug Administration (CFDA), accelerated the regulatory process and has approved Peramivir (under development by the China Academy of Military Medical Science [1]), an injectable NAI for treatment [1,3]. The China's national stockpiles of Oseltamivir and Zanamivir were also reviewed and renewed [3]. The US CDC recently recommended that the clinical benefit was greatest when antiviral chemotherapy was administered within 48 hours of influenza illness onset and treatment with Oseltamivir or inhaled Zanamivir should be started when confirmed cases, probable cases, or contact cases under investigation were recognized, even if more than 48 hours from illness onset and even for apparently uncomplicated illness [9]. Antiviral chemotherapy was recommended as soon as possible for all individuals, even for previously healthy people, and particularly for those considered to be at increased risk of complications associated with influenza such as children aged less than 2 years, adults aged 65 years or over, pregnant women, individuals with certain underlying medical conditions [9]. Decision to start antiviral chemotherapy for outpatients with uncomplicated disease in whom fever was absent and symptoms were nearly resolved should be based on clinical judgment [9]. Patients with severe or complicated illness and hospitalized cases were also recommended treatment with oral Oseltamivir (and not inhaled Zanamivir) for at least 10 days and not recommended inhaled Zanamivir for individuals with underlying respiratory tract diseases such as chronic obstructive pulmonary disease or asthma [9].

Intravenous Zanamivir should be considered for patients who cannot tolerate or absorb oral Oseltamivir although intravenous Zanamivir is an investigational parenterally administered product available by enrollment in a clinical trial or compassionate use under an emergency investigational new drug (EIND) requested to the manufacturer confirmed with the US-Food and Drug Administration (FDA) [9]. Whereas no data are available regarding early NAIs treatment of H7N9 virus-infected individuals, the potential severity of H7N9 virus-associated illness warrants recommending that all confirmed cases, probable cases, and influenza A (H7N9) cases under investigation receive antiviral chemotherapy with a NAIs agent as early as possible [2]. Ingesting woad root ("Banlangan" in Chinese), an example of the traditional Chinese remedies have been suggested by the government officers to kill the influenza A (H7N9) viruses, but at

least one peer-reviewed study indicated that there was little or no effect on NA inhibition while metabolites of the root may inhibit NA with some degrees [1].

Vaccination against Avian Influenza A (H7N9)

Currently, no specific vaccines against influenza A (H7N9) viruses yet exist but now underway in China, Taiwan and the Unites States (US National Institute of Health (NIH), both clinical lots and initial trials [5]) leaded by the WHO GISRS laboratories [2,5] and the WHO Essential Regulatory Laboratory National Institute for Biological Standards and Control (NIBSC) [5] basing on the HA and NA genes of A/Anhui/1/2013-like H7N9 reassorted with the internal genes from PR8 to enhance their growth in eggs [2]. First reports demonstrated that influenza A (H7N9) viruses grew very well in eggs [5]. Contemporary methods will approximately take 5-7 months to produce a marketable product [1,2] whereas attenuated virulence already has resulted in candidate vaccine viruses [2]. There were preliminary antigenic differences of an H7N9 virus when compared with vaccine candidates for Eurasian or North American lineages of H7 subtype viruses [2]. The WHO has prepared six kinds of available H7 vaccines which have the opportunity to prevent the H7N9 virus infections [4].

Currently, the WHO Headquarters is focusing on the establishment of biosafety level (BSL) guidelines to be used for the development and of the human influenza A (H7N9) vaccines [2,5]. These guidelines for uncharacterized candidate vaccine virus strains likely will recommend a high level of biosafety, BSL-3 or BSL-3+ and with a lower level recommended for attenuated strains [5]. There was also a recent report of a rapid epitope-driven vaccine design (within 36 hours) from EpiVax Incorporation [1]. An oral (more mild disease) or injectable (severe disease) anti-influenza formulation, FluCide™ produced by NanoViricides, Incorporation comprises a molecule that mimics the sialic acid receptor of influenza virus (both the mammalian [sialyl-α-2,6-gal-] and avian [sialyl α-2,3-gal-] forms), coating the virus and preventing its attachment to cells whereas phase I trials are pending [1]. Greffex with support from the US NIH claimed that they completely developed an H7N9 vaccine, carrying HA and NA genes on a gutted adenovirus background [1]. Additionally, novel vaccine manufacturing technologies, such as tissue-cell-culture-derived vaccine antigens and recombinant HA may be used [2]. To date, it does not ensure that which vaccine formulations manufacturing are considering, especially whether to include an adjuvant or not [5].

Case Definitions for Avian Influenza A (H7N9) Investigations

The US CDC provides US-based advice to define cases as the following : 1) confirmed case : CDC-certified laboratory tested and positive, 2) probable case : compatible illness, influenza A positive, H1-negative, H1pdm09-negative and H3-negative by RT-rtPCR, and 3) case under investigation : compatible illness with pending, unclear or unknown laboratory confirmation but with (a) recent (10 days or less) contact with a confirmed/probable case or (b) recent travel history to an H7N9 animal or human-positive area [1].

China's Response Strategies and Measures

Significant efforts were made to ensure that the emergency response to the newly identified H7N9 virus was based on laws and regulations, prioritization, principle of transparency and international collaboration [3]. A joint multi-sectoral prevention and control mechanism (JPCM) as well as an inter-regional JPCM had been established at both local and national levels to conduct and coordinate the emergency response to the novel H7N9 virus [3]. At national level, the JPCM conducted by the National Health and Family Planning Commission that consisted of 13 governmental ministries and commissions, including the Ministry of Agriculture, the State Forestry Administration, and the Ministry of Science and Technology [3]. Inter-regional JPCM supported sharing of information and coordinated response among the affected provinces, including Shanghai, Anhui, Zhejiang, and Jiangsu [3]. Different response strategies and guidance had been provided to the different provinces based on the local needs and epidemiological situation [3].

The "Four Earlys" were created which comprised of early detection, early reporting, early diagnosis and early treatment for the operational response to H7N9 viruses [3]. Response measures included close collaboration between public health and animal health sectors, field investigation, enhanced surveillance in humans and animals, risk assessment and communication, clinical management, hospital infection prevention and control, public health interventions, and research [3]. It was likely that most human H7N9virus infections had been associated with contacts with animals or live-bird markets [3,6]. If sustained person-to-person transmission occurs with an increased number of clinically severe cases, health systems are likely to be stressed [6]. The rate of new human infections with H7N9 viruses with onset of clinical manifestations in the following weeks after the closure of live-bird markets in eastern China had substantially decreased, indicating that the primary risk factor was exposure to infected poultry, particularly at live poultry markets [2,3,6] whereas there was no evidence that international spread of H7N9 viruses occurred [6].

It remains to be observed whether H7N9virus infections will follow the same seasonal pattern like other avian influenza viruses such as H5N1 virus which have shown a seasonal pattern in which human cases have been less frequent in summer months and more frequent in winter months [6]. Several technical guidance documents had been provided for epidemiological investigation and surveillance including laboratory testing, patient isolation and treatment, and contact tracing [3]. At present time, investigations have not demonstrated evidence of sustained spread of this novel virus from individual to individual; nevertheless, in a few small clusters of human H7N9 virus infections, the possibility of limited individual to individual spread cannot be excluded whereas the epidemiological investigation of contacts relied on influenza-like symptoms development to trigger collection of clinical samples for laboratory diagnosis [2]. Thus, asymptomatic human H7N9 virus infections resulting from contact with infected cases may have un-detection, and testing of serum samples collected from these cases who contact with confirmed cases will be critical to address this problem [2]. Understanding of the denominator of the total number of human H7N9 virus infections, including asymptomatic, clinically mild, severe, and fatal illness will assist to instruct assessment of the overall severity among the general population [2].

The priority response measures had been specified on the following: 1) field investigations, including source of infection, 2) enhanced surveillance in humans and animals, 3) clinical management, infection prevention and control, 4) risk communication, and 5) Scientific research [3]. In conclusions, response at local and national levels appeared to be effective and excellent and the risk assessment and evidence-based response to H7N9 virus could serve as a model of emergency response to the similar events [3]. Currently, the WHO and member countries remain alert for evidence of events of high significance, including the following:

1) Reassortment with human seasonal or avian A (H5N1) viruses,

2) Virus mutations including those associated with receptor-

binding affinity, antiviral susceptibility, virulence and transmissibility,

3) New human cases and clusters of H7N9 virus infections in China and outside of China, and

4) Person-to-person transmission of human H7N9 virus infections [2].

In its capacity of leading technical agency, the WHO is monitoring the situation very closely, developing and adjusting appropriate interventions in collaborations with it partners around the world [2].

Discussion

It currently remains unknown zoonotic outbreak if the H7N9 virus is being transmitted from wild bird reservoir to poultry with sporadically transmission to humans in multiple unknown locations, most probable the live-bird markets (72% of cases reported some recent contacts with live- poultry and live-bird markets [3]), probably facilitated by the fact that people in China still buy poultry for domestic consumption underwent through both intra- and inter-provincial trading [5] and supported by a reduction in the number of new human cases that associated with the closure of live poultry markets in Shanghai [3] or if the virus has spread to the affected provinces through poultry-to-poultry transmission like scenario in eastern China whereas the novel virus causes mild or no disease in birds and poultry [3,5] and lower pathogenic compared with avian influenza A (H5N1) viruses [5]. This evidence was supported by no detection of bird influenza virus in dead pig specimens from a river in Shanghai [4] and genetically supported by demonstration of a single Arg at the HA cleavage site [3].

While like-birds is the most probable reservoir, reservoir of H5N1 viruses presently is still unknown, but H5N1 viruses are detected in both domestic poultry in some countries and wild birds on occasion [5]. Only domestic birds in some live-bird markets in eastern China were demonstrated H7N9 infections and yet unknown distribution in wild birds whereas H5N1 virus distribution among domestic animals is entrenched and occasionally identified in a limited number of species, including identification in Europe [5]. This novel virus is transmissible among birds and has possibly distributed itself among the poultry populations [5], but currently, demonstrates no evidence of person-to-person transmission [2,4] same as H5N1 viruses [5]. The H7N9 viruses seem more common than H5N1 viruses which are very rare in transmission from animals to humans [5]. Influenza A (H5N1) virus spreading has persisted and evolved over nearly two decades whereas durability as an animal infection of H7N9 viruses is yet unknown [5]. Most laboratory-confirmed cases were reported with underlying chronic medical conditions [2, 5] and progressed to respiratory distress syndrome [2] or severe pneumonia [3] liked most cases of H5N1 virus infections. Most cases had older age range than cases of H5N1 virus infections [2,3,5] and male cases were twice as common as female cases [2,5] whereas there were equal numbers of male and female cases with of H5N1 virus infections and were most common in children and younger adults [5]. This is an unusual and unexplained age and gender distribution in cases with H7N9 virus infections in China [5].

Nevertheless, identification of the virus in any particular species of bird does not necessarily mean that the species is the reservoir for transmission to humans [5]. Whether is being circulating in other animal reservoirs is to be identified yet [5]. Some cases with asymptomatic H7N9 virus infections had been notified whereas asymptomatic H5N1 virus infections were hardly ever [5]. Case-fatality was high (20% [2,5]) among H7N9 virus infected cases, but was very high among H5N1 virus infected cases (60% [5]). Genetically, either E627K [3,5] from some human isolates or D701N demonstrated in PB2 gene in different strains are both markers of mammalian adaptation of H7N9 viruses, such as PB2 Asp701Asn substitution [5,8]. Identification of association between Gln226Leu and Gly186Val substitutions in human virus H7 and increased affinity for α-2,6 –linked sialic acid receptors that found in upper respiratory tract of both humans and other mammals is able to cause severe respiratory infections [3,8]. The presumed incubation period ranged 3 to 8 days [1,8], thus close contacts must be under medical observation at least 10 full days [4,5].

Diarrhea or vomiting was reported approximately 13.5% in a recent study in addition to ILI [7] but most cases resulted in clinically severe illness [6] and multiple organ failure was usual cause of death [2,5]. Lymphocytopenia and thrombocytopenia are likely to be prognostic indicators for ARDS and death in patients with H7N9 virus infections same as H5N1 virus-infected cases [7]. Sputum examination yields positive results higher than the examination results of throat swab specimens [8] likes the results of sputum examination for other microorganisms. Real-time RT-PCR assays are recommended for confirmation of H7N9 virus infections [1-3,5,7]. Influenza A (H7N9) virus resistance to Amantadine and Rimantadine was noted; but, fortunately, the virus still susceptible to both Oseltamivir and Zanamivir [1,3,5,6,9]. A recent study the median time from onset of illness to death was 14 days [7], thus, the chemotherapy with oral Oseltamivir should be started within 48 hours from illness onset for at least 10 days [9].

Although no specific vaccines against H7N9 viruses yet exist in present time, the WHO currently is ongoing the research based on the HA and NA genes of A/Anhui/1/2013-liked H7N9 resorted with the internal genes from PR8 [2]. A promising vaccine was produced by NanoViricides, Inc. which comprises of a molecule that mimics both mammalian' s and avian' s sialic acid receptor of H7N9 viruses [1] but seem to be not ensured whether an adjuvant is included or not [5]. The priority response was set and specified on the following:

1) Field investigations, including source of infection,

2) Enhanced surveillance in humans and animals,

3) Clinical management, infection prevention and control,

4) Risk communication, and

5) Scientific research and the "Four Earlys" (early detection, early reporting, early diagnosis and early treatment) had been effectively used for the operational response to H7N9 viruses in this China' s scenario [3] which can be a model for other countries against the same crisis.

Conclusion

Live-bird markets are the most probable source of H7N9 virus transmission in 2013 China's outbreak. H7N9 virus has lower pathogenicity compared with H5N1 virus. The genotype of H7N9 viruses isolated from humans in China is most likely by ressortment of poultry A (H9N2) viruses in duck. The optimal duration for contacts observation and for chemotherapy with Oseltamivir or Zanamivir is full 10 days while no specific vaccines against H7N9 viruses currently yet exist.

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