UPDATED RAPID RISK ASSESSMENT

Human infection with a novel avian influenza virus, A(H7N9) – China

8 May 2013

Main conclusions and recommendations

Background

On 31 March 2013, Chinese authorities announced the identification of a novel reassortant A(H7N9) influenza virus isolated from three unlinked fatal cases of severe respiratory disease in eastern China; two in Shanghai and one in Anhui province. The WHO Collaborating Centre for Reference and Research on Influenza at the Chinese Center for Disease Control and Prevention (CCDC) had subtyped and sequenced the viruses; they were found to be almost identical and considered to be of low-pathogenic avian origin. This is the first time that human infection with avian influenza virus A(H7N9) has been identified. This is also the first time that a low-pathogenic avian virus caused lethal human infections.

Since then, human cases have continued to be reported, and as of 1 May, there were 128 laboratory-confirmed cases, including 26 deaths reported from eight neighbouring provinces (Anhui, Fujian, Henan, Hunan, Jiangsu, Jiangxi, Shandong, and Zhejiang) and two municipalities (Beijing and Shanghai). One case who acquired his infection in Jiangsu travelled to Taiwan before developing illness.

A few small clusters were detected but almost all cases have occurred sporadically, without obvious epidemiological links. While occasional human-to-human transmission in the clusters cannot be ruled out, there is certainly no confirmed sustained human-to-human transmission. A few mild cases have been detected.

Outside the clusters, more than 3000 contacts of the cases were traced, which did not lead to the detection of additional laboratory-confirmed cases. Genetically almost identical viruses have been isolated from poultry and environmental samples taken from live-animal markets in several provinces almost all of which also reported human cases. The working hypothesis therefore remains that the outbreak is caused by a zoonotic outbreak of reassortant avian influenza virus with low pathogenicity for birds and low transmissibility from birds to humans, but causing severe disease in most of the infected people. The reservoir and source of the viruses in the live-bird markets remain unclear.

The most plausible underlying scenario is of a zoonotic avian influenza that has spread or is spreading in poultry in parts of eastern China. It is a severe threat to humans because of its lethal effect in 20% of human cases and genetic features that have human pandemic potential.

Many questions remain unanswered about this virus and its prevalence, history and behaviour in humans and birds. The numbers of human cases have declined in the last few weeks, especially since live-bird markets were temporarily closed in Jiangsu, Zhejiang and Shanghai. However, the incidence of avian influenza infections in humans usually declines naturally in the spring and summer in the Far East.

Main conclusions and recommendations, continued

EU citizens living or working in China are strongly advised to avoid live-bird markets. A likely scenario involves travellers who get infected in China and import the disease to Europe. Public health authorities should be prepared for the importation of the disease. However, the occurrence of imported cases would not change ECDC's risk assessment.

ECDC's view is that if this virus persists in poultry, it will represent a significant long-term threat, either as a zoonosis or perhaps a pandemic virus. Both eventualities should be prepared for.

Major developments since the first update of the ECDC Risk Assessment of 12 April 2013

- The number of cases of human A(H7N9) has risen rapidly to over 120 in a short period of time.
- There has now been a slowdown in the numbers of new human cases detected in China per week.
- The case-fatality rate has remained at about 20%.
- Although there are a few small family clusters, almost all of the cases represent sporadic human infections without epidemiological links and no detectable infections among contacts.
- The geographic distribution has expanded to eight neighbouring provinces and two municipalities in China; this may represent discovery of cases and infection in birds rather than actual spread.
- The first reported travel-related case is a case in Taiwan who was infected in Jiangsu.
- Exposure to live poultry and environmental contamination in markets is suggested to be an important risk factor for human infection.
- There are a number of virological markers of adaptation to mammalian hosts, but there is no evidence of efficient human-to-human transmission or chains of transmission.
- A very few mild or asymptomatic infections have been detected, mostly in children.
- Cases were predominantly in older males; the reasons for this are not understood.
- Important differences are becoming apparent between the two avian influenza strains A(H7N9) and A(H5N1), notably the low pathogenicity of A(H7N9) in poultry.
- In China, many samples from birds were tested but less than 1% were positive. However, it is acknowledged that the animal testing methods and strategy have not yet been validated against the new viruses.
- Samples of the virus have been distributed to international WHO Collaborating Centres for Reference and Research on Influenza for further investigation.
- The selection of viruses suitable for vaccine development has started, also in Europe. The United States authorities signalled that they are going to develop some experimental vaccines (clinical lots) for early clinical trials.
- International groups working with the Chinese authorities have completed in-country assessments of the situation and the response for WHO and OIE.
- In Europe, testing capacity is being established in national influenza centres and public health laboratories.
- A case-finding strategy and case definition was published by ECDC.

Recommendations for the EU/EEA

- EU/EEA citizens working in or visiting China should avoid visiting live-bird and animal markets ('wet markets') because of the potential presence of avian influenza viruses that are pathogenic to humans in these markets. They should also avoid direct contact with bird and animal faeces, untreated bird feathers and other animal and bird waste, and they should follow basic hand hygiene rules, e.g. hand-washing with soap and the use of alcohol-based hand rubs.
- Specific food safety recommendations for the EU are not required for the A(H7N9) outbreak. Longstanding advice that chicken and eggs should be properly cooked remains relevant.
- As there is no evidence of efficient human-to-human transmission at this stage, tracing of contacts of passengers that are symptomatic during a flight home from China and chemoprophylaxis for them is not recommended, but could be considered if the case is later confirmed.
- Public health authorities should apply the case-finding strategy developed for A(H7N9) and the EU case definition.
- National public health authorities and infection control managers should alert and remind clinicians and healthcare workers of standard guidance for investigation, infection control and contact tracing around cases of severe acute respiratory infections.
- Public health authorities should investigate all clusters of severe respiratory infections and infections in healthcare workers who have been caring for patients with severe acute respiratory disease, considering a number of pathogens, not just A(H7N9).
- Deferral criteria for blood safety should stay unchanged, i.e. persons with acute febrile illness should not donate blood until after they have fully recovered.

Main conclusions and recommendations, continued

- National authorities should make preparations for the occurrence of confirmed cases in the EU/EEA Member States; authorities should consider active tracing, follow-up of close contacts and people who may have shared the same exposure, as well as antiviral prophylaxis following standard guidance and protocols.
- Diagnostic and reference laboratories in the EU should continue to use their current generic RT-PCR assays for influenza A for screening and testing of possible cases if those tests are based on highly conserved internal gene sequences (e.g. in the M gene segment). The match of primers and probes to the published sequences of the A(H7N9) virus should be checked and validated with available positive control material.
- Clinicians and laboratory specialists should be reminded to consider the possibility of animal influenza infection in persons with severe acute respiratory disease who have, in the previous 10 days, been in China and other countries with circulating animal influenza viruses pathogenic to humans.
- Influenza A virus isolates that cannot be sub-typed at national reference laboratories must be sent rapidly to the WHO Collaborating Centre for Reference and Research on Influenza based in Europe.
- Member States should not report cases under investigation for A(H7N9) internationally before confirmation. However, clinicians and laboratories may need to notify their national authorities about cases under investigation in accordance with national guidelines.
- Any confirmed case being diagnosed in the EU/EEA area should be immediately reported to international authorities by national contact points through the Early Warning and Response System (EWRS) and to WHO under the International Health Regulations (2005). Reporting through EWRS qualifies as IHR notification and avoids double reporting.
- Some population-based age-specific serological surveys of likely immunity to A(H7N9) should be undertaken using CONSISE protocols or their national equivalents in a number of populations.
- In accordance with WHO guidance, EU/EEA Member States should not implement travel or trade restrictions for countries with A(H7N9) transmission.
- Member States should liaise with their national animal health agencies to ensure timely exchange of surveillance data for A(H7N9) and consideration should be given for how joint 'One Health' surveillance might be undertaken.
- EU/EEA countries should continue to review and update their pandemic preparedness plans, not only for A(H7N9) but for any novel virus; this process should include the lessons learnt from the 2009 pandemic and new guidance from WHO.

Source and date of request

Planned revision by ECDC, 29 April 2013.

Public health issue

The aim of this document is to assess the risk associated with the outbreak of avian influenza A(H7N9) to public health in the EU and to EU citizens, to anticipate likely future developments, and to make limited recommendations for Europe. This document summarises the situation with regard to the novel avian influenza A(H7N9) (as of 6 May 2013) and focuses on developments after the publication of ECDC's Rapid Risk Assessment of 3 April 2013 [1], updated 12 April 2013 [2].

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External experts consulted and acknowledgements

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This analysis would not have been possible without the virological and molecular data made available in the GISAID database [3] by the WHO Collaborating Centre for Reference and Research on Influenza at the Chinese Center for Disease Control and Prevention; the Harbin Veterinary Research Institute, the Ministry of Agriculture, China; and the Hangzhou Center for Disease Control and Prevention, Hangzhou, China [4-6]. The GISAID database is hosted by the federal government of Germany.

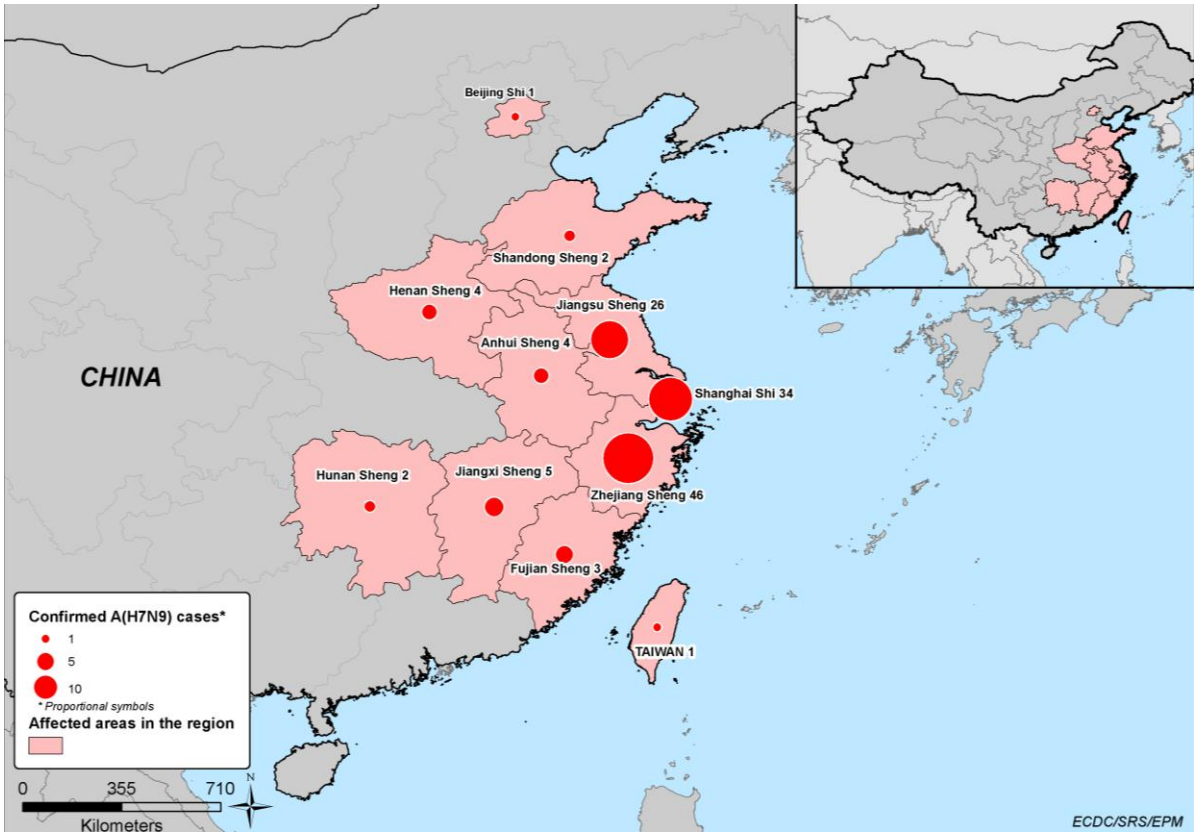
Event background information

Human epidemiology

On 31 March 2013, the Chinese authorities announced the identification of a novel influenza (H7N9) virus infection in three seriously ill people who subsequently died. These cases occurred in the Chinese provinces of Shanghai and Anhui [1]. The Chinese CDC has released specific avian influenza A(H7N9) tests which were distributed across the country – via CCDC's influenza laboratory network – to major hospitals, research agencies, and over 400 influenza monitoring sites. Epidemiological data are released weekly, and the epidemiological information presented below mostly represents what was available on 1 May 2013.

As of 1 May, there have been 128 laboratory-confirmed cases of influenza A(H7N9) virus, distributed over 39 prefectures/districts in 10 provinces/municipalities (Figure 1), 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 case, 0 death), Henan (4 cases, 0 death) and Hunan (2 cases, 0 death). There has been one travel-related case diagnosed in Taiwan, who was infected in Jiangsu Province [7-10]. Poultry density is very high in this part of China and the extensive poultry trade is considered likely to have contributed to the distribution of the virus [8, 9]. While this represents an increased number of provinces or municipalities compared with ECDC's last risk assessment, it does not necessarily represent a spread of the infection in birds or people. Equally, it could be explained as revealing of incident infections following the increasing availability of testing, awareness among the public and clinicians, and declarations by the authorities that those seeking care will not be financially penalised by hospital costs.

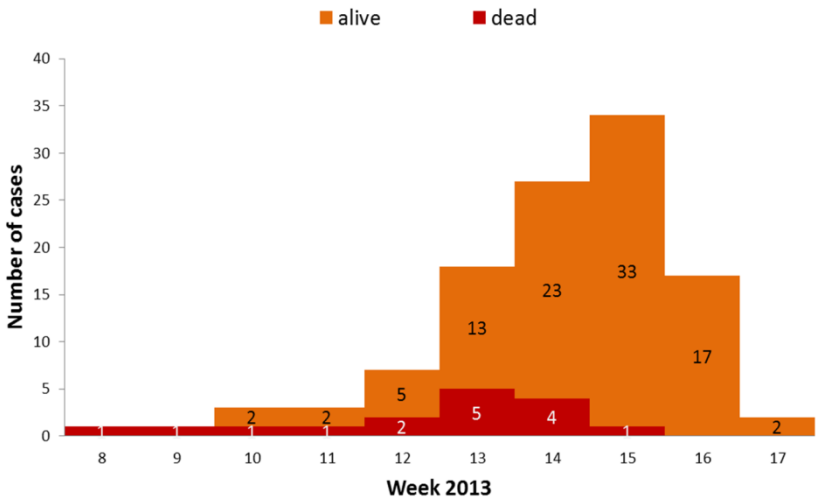
Figure 1. Distribution of cumulative number of avian influenza A(H7N9) human cases, 19 February – 1 May 2013 (N=128)



Data sources:
Chinese MoH, http://www.moh.gov.cn/
China CDC, http://www.chinacdc.cn/en/
WHO Disease Outbreak News, http://www.who.int/csr/don/en/

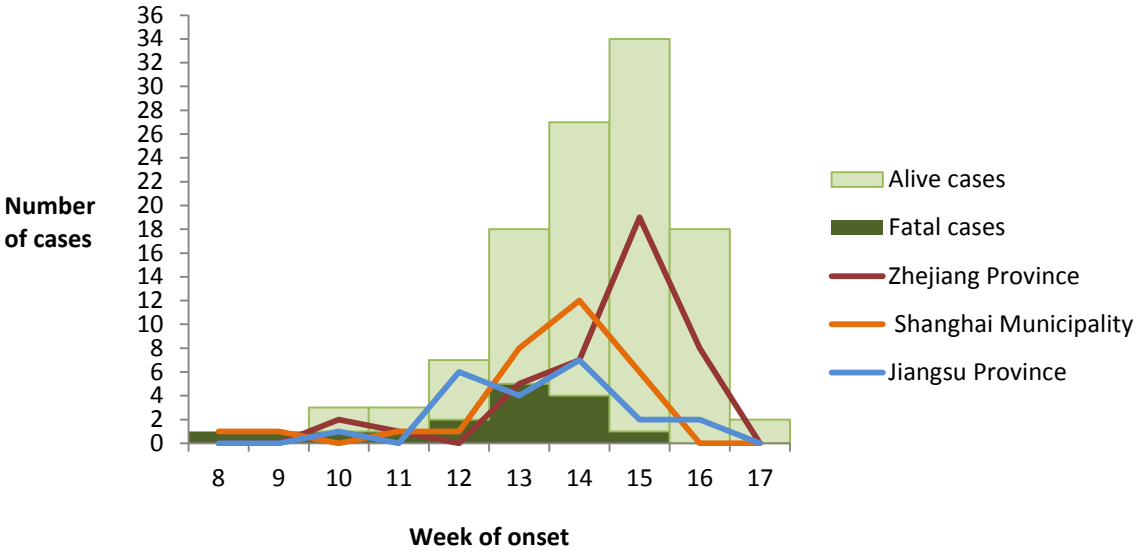
The date of onset ranges from 19 February to 27 April. Looking at the epidemic curve (Figure 2), no acceleration in numbers can be detected, despite the widespread availability of testing and the increased care seeking reported in Shanghai Municipality and Jiangsu and Zhejiang Provinces. If anything, and even allowing for reporting delay, there has been a decline in incidence by date of onset (Figure 2).

Figure 2a. Distribution of influenza A(H7N9) cases by week of onset of symptoms, as of 1 May 2013 (N=113 cases with onset data)



** No onset date for 15 cases, missing fatalities for 10*

Figure 2b. Distribution of influenza A(H7N9) cases by week of onset of symptoms, displaying Shanghai municipality and Jiangsu and Zhejiang Provinces, 1 May 2013 (N=114 with onset data)



** No date of onset for 14 cases, 10 fatalities are missing*

Data sources:
Chinese MoH, http://www.moh.gov.cn/
China CDC, http://www.chinacdc.cn/en/
WHO DONs, http://www.who.int/csr/don/en/

Of the 128 confirmed cases, 26 (20%) died and 26 are known to have recovered. Only three cases were confirmed among children. The overall age of the cases ranged from 4 to 91 years, with a median age of 62 years. Of the confirmed cases, 87 (68%) were male and 36 (28%) female; gender was not stated for five cases. Of 71 cases with available data, 54 (76%) had a previous underlying medical condition or conditions. Most of the confirmed cases were hospitalised, which, in a few cases, was only for infection control purposes.

For 82 confirmed cases, exposure information is available, stating that 63 (77%) of them have been exposed to live animals [10]. The incubation period is difficult to state at present as there have been few clear exposure events. The first published analysis has a median incubation period for all patients of six days, with a range of one to 10 days [10]. ECDC used this as the basis for the 10 days recommended in its case finding algorithm [13]. The transmission of infection to humans is probably facilitated by the fact that many people in parts of China still buy live poultry for domestic consumption. However, other possible animal reservoirs remain to be investigated. There is no information available as yet from analytical studies of risk factors for infection such as case control studies or behavioural investigations.

Close to 3 000 contacts have been followed-up and only four are reported to have developed symptoms, as part of three small family clusters. In Shanghai, two family clusters were identified with two confirmed and one suspected A(H7N9) cases. One of these persons recovered but the other two died in February from respiratory failure. The second probable family cluster includes a husband and wife from Shanghai, both confirmed cases. A third family cluster was identified in Jiangsu, with one confirmed and one suspected case. Both were hospitalised in a critical condition. In the first two clusters, it was not possible to determine whether there had been limited person-to-person transmission or whether those infected were exposed to a common source. This has often been the case with outbreaks of influenza A(H5N1), which causes severe illness in humans.

The human influenza surveillance system in China is not reporting an overall increase in influenza virus detection or atypical pneumonia cases in the most recent reporting period. Although there have been increased reports of influenza-like illness (ILI) in sentinel systems in the first affected areas, analyses of the underlying viruses have found hardly any A(H7N9) but a number of seasonal viruses. This increase in ILI is therefore probably explained by increased care seeking and testing encouraged by the authorities. Hence, the available epidemiology is not compatible with efficient human-to-human transmission of A(H7N9) [14].

In making comparisons between A(H7N9) and A(H5N1) in humans, there are clear differences, most notably a considerably greater number of human A(H7N9) cases presumed to come from animals or the environment over a matter of weeks (over 100 confirmed A(H7N9) human cases), compared with only 43 human cases of A(H5N1) in the mainland of China between 2003 and 2013 [14]. There is also an unusual and as yet unexplained age and gender distribution in China: cases of A(H7N9) are considerably older than for A(H5N1), and male A(H7N9) cases are more than twice as numerous as female cases for A(H7N9), while there were roughly equal numbers for A(H5N1) [13, 14]. Prior exposure to influenza 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, visiting markets or care seeking [17].

Table 1. Preliminary comparison of A(H5N1) and A(H7N9) in humans, as of 29 April 2013

Item	A(H5N1)[a-c]	A(H7N9) [10]
Human epidemiology	Equal numbers of male and female cases. Most common in children and younger adults	An older age range than A(H5N1); male cases are twice as common as female cases
Observed case-fatality ratio	Very high, around 60%	High, around 20%
Disease spectrum	Commences as influenza-like illness and then severe respiratory disease and may progress to multi-organ failure; mild cases very uncommon	Commences as influenza-like illness and then severe respiratory disease and may progress to multi-organ failure; some mild cases
Asymptomatic infections	Hardly ever	Some observed
Transmission from animals to humans	Very rare; mostly observed in association with poultry die-offs or substantial environmental exposure	Seems more common than for A(H5N1). Initial suspected risk factor is direct or indirect contact with live poultry in live-bird markets
Human-to-human transmissibility	Has happened occasionally, following prolonged close contact, especially in the context of caring for sick persons	Cannot yet be excluded
Sustained human-to-human transmission	Nil	Nil

a) World Health Organization (WHO). H5N1 avian influenza: Timeline of major events. Geneva: WHO; 17 Dec 2012. Available from: http://www.who.int/influenza/H5N1_avian_influenza_update_20121217b.pdf

b) Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus. Update on Avian Influenza A (H5N1) Virus Infection in Humans. NEJM 2008; 358:261-273. Available from: http://www.nejm.org/doi/full/10.1056/NEJMra0707279

c) WHO Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003–2013, 26 April 2013. Available from: http://www.who.int/influenza/human_animal_interface/EN_GIP_20130426CumulativeNumberH5N1cases.pdf

Clinical aspects and spectrum of disease

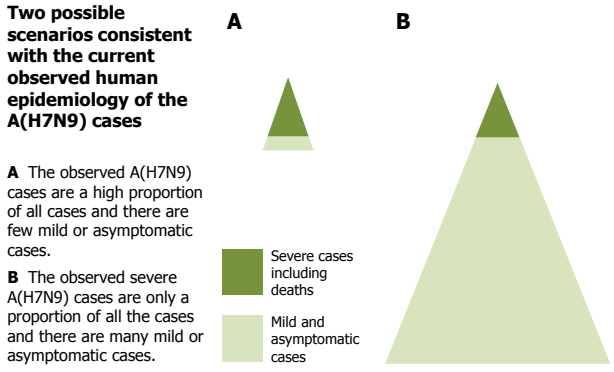
Most of the cases have presented with influenza-like illness. Unlike other A(H7) avian influenza infections, initial conjunctivitis has not been a feature reported for the first cases [10]. Many of the cases then progressed to pneumonia, with a significant proportion developing acute respiratory distress syndrome. Respiratory failure and multi-organ involvement was the usual prelude to death, which occurred in about 20% of the cases, in a manner reminiscent of A(H5N1) disease [14].

Figure 3 outlines two potential scenarios:

- A (observed cases are a high proportion of cases and there are few mild cases); and
- B (the observed cases are only a small proportion, and there are many unobserved mild cases).

There have been some mild and even asymptomatic cases, mostly among younger patients, and at present the full spectrum of disease is unclear (Figure 3) [14]. Currently, the near total absence of cases among contacts of the reported cases, despite extensive contact tracing, seems to speak somewhat against Scenario B. However, the testing of contacts has relied mostly on nasopharyngeal swabs, and it is not clear that these are optimal for virus detection. Also, if most cases are sporadic cases among contacts, they would also be very low in number. In order to resolve this, serological surveys are needed among the contacts, using tests validated against the new viruses and CONSISE protocols (or their national equivalents) [16, 17].

Figure 3. Two possible scenarios consistent with the current observed human epidemiology of A(H7N9) cases



Animal infections

Animal surveillance is ongoing around the cases and the Chinese Ministry of Agriculture has notified the World Organization for Animal Health (OIE) about the detection of some genetically similar A(H7N9) isolates from birds [20]. Animal infection data are hard to interpret as information on whether the tests used have been validated against the novel avian influenza viruses is unavailable [21]. As of April 26, national and provincial avian influenza reference laboratories in China have collected 390 628 samples for virus testing from 13 014 surveillance sites around the country, including 2 587 live-poultry markets, 337 poultry slaughterhouses, 8 808 poultry farms, 341 wild birds habitats, 277 pig slaughterhouses, 31 pig farms, and 633 other sampling spots. It is reported that 218 897 samples were tested, including 150 837 serology samples and 68 060 pathology samples. Of the 68 060 samples, only 46 tested positive for the virus, resulting in a positive rate of 0.07% [22]. As we do not know how the sampling and testing are related to the human cases and due to a lack of information on animal test validation, it is difficult to interpret these data at present.

Of those animals tested positive, almost all (44) were from 14 live-poultry markets in east China's Jiangsu, Zhejiang and Anhui provinces, central China's Henan province, and the Shanghai Municipality. The other two cases were from Jiangsu, including one wild pigeon and one racing pigeon collected at a household farm. Positive samples were also reported from Jiangxi, Shandong, and Guangdong Provinces, three provinces that so far have not reported human cases [23]. No samples collected from source poultry farms feeding into the markets have as yet tested positive for the A(H7N9) strain of avian influenza, and the virus has not been detected in pigs [22]. However, testing of the source farms is incomplete and it would only require a few of the many farms sending infected poultry to the live-bird markets to have these infections amplified in the markets; this would also lead to environmental contamination [12]. In areas like Shanghai, the number of animal infections is low compared with the numbers of human infections. This could indicate another animal host or a virus in transition, i.e. an avian virus adapting to human hosts. However, the possibility of an underestimate of the true numbers of infections in birds due to a lack of test sensitivity could not be excluded [21].

At least three provinces closed live-poultry markets in urban areas. The Chinese Ministry of Agriculture (MoA) also reported additional control measure to the OIE [20], for example 'stamping-out' control measures in affected poultry markets, as was noted by a joint Chinese-WHO inspection team which visited Shanghai in April 2013 [24]. Market closures have been associated with a decreased number of human cases in those localities [14]. However, it would be premature to draw any conclusions because the weekly numbers of human A(H7N9) infections are now declining (Figures 2a–2b), and avian influenza infections in reservoir hosts may have a seasonal pattern as well. A comparison of the known characteristics of A(H5N1) and A(H7N9) may reveal many unknowns for the latter and also one important difference (Table 2): the low or zero pathogenicity of A(H7N9) with regard to its avian hosts, which means that potentially these viruses can spread silently in poultry, and the human disease and death may be the first indication of infections in birds. The true characteristics of the infections in a range of animal species will now have to be determined experimentally in the OFFLU laboratories, particularly with regard to low/zero pathogenicity; optimal testing for surveillance; and infection risk for those working with, or being close to, poultry.

Table 2. Preliminary comparison of A(H5N1) and A(H7N9) in birds, as of 29 April 2013

Item	A(H5N1)[a-c]	A(H7N9)
Reservoir of infections	Unknown, but viruses are found in both domestic poultry in some countries and wild birds on occasion	Unknown as yet
Distribution in domestic animals	Entrenched in domestic poultry in a limited number of countries	Only detected in domestic birds in some live-bird markets in eastern China
Distribution in wild birds	Occasionally detected in a number of species, including detection in Europe	Unknown as yet
Pathogenicity in poultry	Highly pathogenic – detected by flock die-offs	Low or zero pathogenicity in poultry, no flock die-offs. Human cases may be the first indication of poultry infections
Durability as an animal infection	Has persisted, spread and evolved over nearly two decades	Unknown; has only been reported since early 2013

a) World Health Organization (WHO). H5N1 avian influenza: Timeline of major events. Geneva: WHO; 17 Dec 2012. Available from: http://www.who.int/influenza/H5N1_avian_influenza_update_20121217b.pdf

b) Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus. Update on Avian Influenza A (H5N1) Virus Infection in Humans. NEJM 2008; 358:261-273. Available from: http://www.nejm.org/doi/full/10.1056/NEJMra0707279

c) WHO Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003–2013, 26 April 2013. Available from: http://www.who.int/influenza/human_animal_interface/EN_GIP_20130426CumulativeNumberH5N1cases.pdf

Virological information

Initial characterisation of the viruses was carried out by the WHO Collaborating Centres for Reference and Research on Influenza in Beijing, and additional genetic analyses were carried by the WHO Collaborating Centres for Reference and Research on Influenza in Atlanta, London, Melbourne and Tokyo. A first summary was published on the WHO Regional Office for Europe website [25]. However, further publications have now come from Beijing and Tokyo and a summary from Atlanta [4, 26]. Detailed genetic sequence data from human, avian and environmental isolates of A(H7N9) viruses were published at intervals, primarily on the GISAID website, but also in the INSDC databases. These are the basis for subsequent analyses with complex arguments suggesting that multiple reassortment events have taken place, potentially involving wild birds [3, 6, 26].

The outbreak virus is a reassortant avian influenza A virus in which the six RNA segments encoding the internal proteins are closely related to avian A(H9N2) viruses recently isolated from poultry in China. The segment encoding haemagglutinin (HA) belongs to the Eurasian A(H7) avian influenza virus lineage and the segment for neuraminidase (NA) is most similar to avian H11N9 and H7N9 viruses. However, the nearest matches found for the HA and NA are considerably less closely related than for the six internal-gene RNA segments. This gene constellation makes the outbreak strain different from previously isolated avian influenza A(H7N9) viruses, including those reported in birds in Europe. The sequence diversity observed between isolates of the novel influenza A(H7N9) virus – in combination with a comparison of historical data – suggests that the immediate ancestral viruses that contributed the H7 and the N9 genes remain unknown and that substantial circulation of the virus must have occurred in birds before the recent detection in humans [27]. The reservoir for the novel virus infecting humans remains unknown, but the virus has been detected in domestic birds in live markets in eastern China.

Genetic markers associated with high pathogenicity in poultry have not been detected, but this finding requires further confirmation by intravenous pathogenicity index testing in chickens, which is underway. Low or zero pathogenicity in poultry does not necessarily indicate low pathogenicity in humans: but these viruses are the first low-pathogenicity viruses that have caused severe human disease. The substitution Q226L in the haemagglutinin gene (HA) is present in almost all the human and non-human H7N9 isolates. This substitution has been associated with reduced binding to avian-like receptors, with sialic acids linked to galactose by α-2,3 linkages, which are found in the human lower respiratory tract. This 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 located in the upper airways of humans and other mammals. Experimentally, the Q226L in HA has also been shown to be associated with increased evidence of respiratory transmission between ferrets of A(H5N1) avian influenza viruses [27, 28]. Ferrets are frequently used as a model for how animal influenza viruses will behave in humans [31]. Also noteworthy is the deletion of a stalk region of the NA protein frequently observed upon transfer of viruses from wild fowl to poultry or mammals. Substitutions in the PB2 gene are present and these are known to enhance the replication of avian influenza viruses in mammals. In different strains, either E627K or D701N have been found in PB2, which are both markers of mammalian adaptation [4-5]. An M gene marker S31N for adamantane resistance was found in the first three human isolates [4]. It is therefore anticipated that the viruses will be resistant to amantadine and rimantadine. These two antivirals are no longer in use in Europe. The WHO Collaborating Centre in Beijing has confirmed that A(H7N9) is sensitive to oseltamivir and zanamivir in phenotypic tests [4].

Diagnostics for influenza A(H7N9) virus in humans

Based on sequence analysis, it is expected that the generic RT-PCR assays for influenza A virus that are based on highly conserved viral gene sequences, e.g. in the M gene, will detect the novel virus. Diagnostic and reference laboratories in the EU should therefore continue to use their current generic RT-PCR assays for influenza A virus for screening and testing of possible cases if those tests are based on highly conserved internal gene sequences and have been confirmed *in silico* to fit the sequence of the A(H7N9) virus. Clinically validated assays that specifically detect A(H7N9) viruses are reported to be available in several European countries. Additionally, a couple of countries with frequent H7 poultry outbreaks have assays available that *in silico* are fit to subtype the A(H7N9) virus. According to a survey conducted in July 2011, many national influenza reference laboratories are using PCR tests that likely detect this novel virus and should therefore be reasonably well prepared [32].

In the diagnostic laboratory assays, the novel viruses should be detected as positive for influenza A virus, and negative for influenza B, A(H1), A(H1)pdm09, A(H3) and A(H5) viruses. Hence, A(H7N9) viruses are expected to be classified as un-subtypeable influenza A. Laboratories with experience of A(H7) sub-typing will be able to subtype the novel viruses with their existing H7 primers and probes.

It is standard procedure to send influenza A virus isolates or clinical samples that cannot be subtyped at the national reference laboratory to a WHO Influenza Collaborating Centre for characterisation, as was done in China with the first A(H7N9) isolates. In Europe, the WHO Collaborating Centre at the National Institute for Medical Research in London receives such isolates, as do other European National Influenza Centers. All EU/EEA Member States are expected to urgently send un-subtypeable A viruses and subtyped A(H7) viruses to the Collaborating Centre in London for further characterisation.

To assist European laboratories in verifying and ensuring their diagnostic capability to detect and identify avian influenza A(H7N9) virus, ECDC jointly with CNRL and the WHO Regional Office for Europe released a technical briefing note on Diagnostic preparedness in Europe for detection of avian influenza A(H7N9) viruses [33]. The briefing note provides:

- a list of laboratory preparedness considerations to ensure European-wide diagnostic capability;
- an update on current methods used for molecular detection of human infection with avian influenza A(H7N9) virus by RT-PCR;
- a table of H7 HA assay validation criteria;
- information on positive controls for RT-PCR assays.

Most EU/EEA countries have already requested and received positive control material for the validation of their A(H7N9) detection assays.

Support to national influenza laboratories for shipment of samples for characterisation at the WHO Collaborating Centre for Reference and Research on Influenza in London takes place routinely though the WHO Global influenza Surveillance and Response System. Additional support is available for shipping through the European-Commission-funded Joint Action on Quality Assurance Exercises and Networking on the Detection of Highly Infectious Pathogens (QUANDHIP Joint Action). WHO is currently producing guidance on how to handle clinical material (at BSL-2) and virus propagation (at BSL-3), which is needed both in diagnostic laboratories and in laboratories developing vaccine strains.

Vaccines against influenza A(H7N9)

Influenza candidate vaccine virus strains are usually developed in a few laboratories working with the WHO Global Influenza Surveillance and Response System (GISRS) and then shared with all vaccine manufacturers. It is so far unknown whether current and available H7 candidate vaccine viruses will provide cross-protection against the new influenza A(H7N9) or if new candidate vaccine viruses are needed. There is, however, no guarantee that older H7 vaccine viruses will be effective for protection against this new virus [25].

New candidate vaccine viruses based on the published genetic sequences of influenza A(H7N9) virus and using reverse genetics technology have been developed by several WHO collaborating laboratories, including the WHO Essential Regulatory Laboratory National Institute for Biological Standards and Control (NIBSC). In addition, attempts to develop candidate vaccine virus strains using the classical reassortment technique (best option for egg culture used by most manufacturers) are ongoing. First reports on virus growth of the new influenza A(H7N9) viruses state that they grow very well in eggs.

Contracts between the US government and vaccine manufacturers ensure that candidate vaccine virus strains developed either by researchers or manufacturers are open-source and can be freely shared with all stakeholders. In a media briefing by the US CDC on 5 April 2013, the US efforts for development of a candidate vaccine strain were presented [34]. These include the development of so-called clinical lots and initial trials, which will be conducted under the US National Institutes of Health.

The EU Vaccine Task Force on Influenza (European Commission, European Medicines Agency – EMA, European Food Standards Authority, and ECDC) has been meeting to consider several pending issues and receive briefing

from WHO and NIBSC. The main regulatory work at the EU level will be under EMA and the Commission. As mentioned above, WHO Headquarters is currently focusing on the establishment of biosafety level (BSL) guidelines to be used for the development and production of human influenza A(H7N9) vaccines. The guidelines are expected to be finalised in May 2013. It is likely that these guidelines for uncharacterised candidate vaccine virus strains will recommend a high level of biosafety, BSL-3 or even BSL 3+, with a lower level recommended for attenuated strains. In addition, WHO HQ is undertaking regular telephone conferences with regulatory agencies throughout the world to facilitate evaluation and authorisation of new candidate vaccines; there are also plans to reach out to vaccine manufacturers to assess the production capacities for H7N9 vaccines. As of now, it is unclear which vaccine formulations manufacturers are considering, in particular whether to include an adjuvant or not.

Human and animal surveillance in Europe

Surveillance for respiratory infections in humans

The travel-related case in Taiwan who was infected in mainland China shows how easily an infected person could spread the virus to other regions [7, 35]. As weekly case numbers are declining, ECDC does not necessarily expect imported cases in the immediate future but considers it important to prepare for this eventuality by stepping up disease surveillance. ECDC, with input from Member States, developed a case-finding algorithm and a case definition for surveillance and reporting of patients infected by the avian influenza A(H7N9) virus in EU/EEA Member States [13]. ECDC is currently developing a platform for the reporting of cases, and there are protocols available from CONSISE and national authorities for the investigations around cases. Agreed protocols for clinical investigations were prepared by the ISARIC group and are endorsed by WHO and ECDC [37].

Surveillance and control of low- and high-pathogenic influenza in poultry and other captive birds in the EU

At this stage of the investigations in China, animal testing is yet to be validated and the data are not complete enough to understand the nature of the animal reservoir or how poultry and possibly other animals become infected. Equally unclear are the routes of transmission between poultry and humans or possibly wild birds to humans. The increase in human cases and the expansion from three to ten affected provinces and municipalities in a month suggests that this virus has been circulating for some time in birds and has only come to light through testing of infected humans. This makes it difficult to determine the true extent of infection in birds in China. It is also impossible to calculate the risk of spread of the virus to Europe. It is worth noting that it took A(H5N1) nine years from its first detection in southern China to be detected in wild birds coming to Europe in any significant numbers [38].

EU animal health legislation [39] requires the control of outbreaks in poultry and other captive birds of highly pathogenic and low pathogenic avian influenza viruses of the H5 and H7 subtypes. Suspected and confirmed presence of infection in poultry and other birds must be immediately notified to the competent animal health authority. EU Member States should carry out surveillance programmes in poultry and wild birds [40] in order to detect the circulation of avian influenza viruses according to harmonised guidelines [41].

The Animal Health and Veterinary Laboratories Agency (AHVLA) is the international reference laboratory for avian influenza, recognised by the European Union and networking with the national reference laboratories for avian influenza in Member States. AHVLA has confirmed the predicted utility of EU-recommended PCR protocols for the detection of the H7N9 virus (both H7 and M gene assays) based on sequence comparison of the H7N9 viruses from birds in China. Furthermore, preliminary data indicate that the H7 antigens used in the mandatory European serological survey in poultry for H5/H7 viruses are suitable for the detection of antibodies to H7N9, at least at flock level.

The Food and Agriculture Organization of the United Nations (FAO) is producing guidelines for risk-based surveillance strategies to investigate the presence of infection along the market chain, with the purpose of informing the control strategies and provide data to reduce uncertainty.

EU legislation regarding import of live poultry from China

Importation to the EU of live poultry, their day-old chicks and hatching eggs, and birds other than poultry (captive birds such as parrots, finches and ornamental birds) from China is not authorised [42].

Discussion

Currently, the most likely scenario in China and therefore worldwide is that this remains a zoonotic outbreak in which the virus is transmitted sporadically to humans who are in close contact with the animal reservoir. The new virus causes mild or no disease in birds, but is transmissible among birds and has possibly distributed itself in the poultry populations in an unknown area of eastern China, and perhaps more extensively. Whether it is circulating in other animal reservoirs is yet to be determined. It also remains unknown if the virus is being transmitted from a

wild bird reservoir to poultry in multiple locations or if the virus has spread to the affected provinces through poultry-to-poultry transmission. People are probably being occasionally infected through exposure to (most probably) infected poultry or contaminated environment at live-bird markets [21]. The exact route of transmission to humans is yet unknown. Several species of poultry and other birds are sold in China's live-animal markets, and once introduced, the virus is likely to spread across species and contaminate the environment. Live bird markets provide an environment for amplification and maintenance of the H7N9 virus [21]. However, detection of the virus in any particular species of bird does not necessarily mean that the species is the reservoir or is responsible for transmission to humans.

There has been a reduction of human cases in China since week 15 by week of onset (Figures 2a and 2b). This seems to follow the closure of live poultry markets in the Shanghai Municipality and Jiangsu and Zhejiang Provinces. However, this may be coincidental since the incidence of avian influenza cases often declines in the Far East in the spring and summer. Also, it has to be realised that this intervention will not be possible in many parts of China where there are not alternative safer routes of poultry processing. This decline does not mean that the problem is going away or coming under control [42]. It is significant that the joint Chinese-WHO inspection team concluded that only a long-term, cross-sectoral and scientifically based control programme will defeat or at least contain this serious influenza threat [43]. There may also be a role for A(H7N9) vaccines in poultry in affected areas, as there has been for H5N1 in China.

Previous A(H7) infections in humans have tended to be mild [41-43]. The exception is one death during a large outbreak in poultry in the Netherlands involving highly pathogenic avian influenza A(H7N7) virus [44, 45]. The death in the Dutch case was associated with an E627K substitution in PB2 of the H7 influenza virus [44]. The same E627K substitution has been associated with high virulence, host range adaptation and airborne transmission in H5 viruses though the significance of its presence in A(H7N9) is yet to be clarified [46-48]. Whether this substitution has the same effect in the A(H7N9) virus as in the H5 and H7 viruses is unknown. It may be significant that six out of eight A(H7N9) viruses isolated from humans also have this E627K substitution, and that the remaining two human isolates carry another substitution (D701N) that has been reported to have a similar effect as E627K [6, 49-51]. The absence of the PB2 E627K substitution in the non-human isolates sequenced to date may indicate either that the change occurs in the infected individuals, or that another subset of the virus that has not yet been sequenced is circulating in animals and causes infections in humans. As there is no indication for the latter from the data so far, the current prevailing hypothesis is that these are mutants arising during human infection.

There are reasons for concern over human infections with A(H7) viruses in general [55]. This reassortant virus harbours the internal genes derived from avian A(H9N2) viruses, for which laboratory studies with animals have suggested that they have pandemic potential [53]. LPAI A(H9N2) virus infections of humans have usually resulted in uncomplicated influenza illness, but one case of lower respiratory tract disease in an immunocompromised adult has been reported [57]. As A(H7N9) viruses are considered to have some pandemic potential, these virological characteristics must always be considered in combination with information on their epidemiology and clinical expression in humans [58].

ECDC threat assessment for the EU

The emergence of a novel reassortant avian influenza virus capable of causing severe disease in humans is a significant public health development. The rapid notification by Chinese authorities under the International Health Regulations has been commendable, and the continued communication of the findings of outbreak investigations has facilitated the assessment of the risk to human health from this outbreak in Europe as elsewhere, and it is essential that this practice continues [59, 60].

This is the first time that human infection with influenza A(H7N9) virus has been identified and the first time that human infection with a low pathogenic avian influenza A virus has been associated with a fatal outcome. A(H7N9) may be more transmissible from birds to humans than A(H5N1) and there are a number of worrying virological features. If the virus is able to spread further in birds in China, there is also the risk it will change to be highly pathogenic for birds, as other low-pathogenicity viruses have done. This could threaten food security, an eventuality that is causing concern in some veterinary authorities in China who consider that the infection may already be more widespread in poultry than it appears and difficult to control [61]. As a result of these facts, this particular virus has been identified by the Chinese authorities and WHO as one of the most dangerous animal influenza viruses to date [43]. This is a view endorsed by other international experts familiar with avian influenzas in the Far East [57, 59]. One caveat here is the appreciation that with technical advances, improved surveillance and increased transparency, alerts like this one may become more common in the future [59, 60].

The risk of the disease spreading to Europe via humans in the near future is considered low. However, it is likely that people presenting with severe respiratory infection in the EU and a history of potential exposure in the outbreak area will require investigation in Europe. It is also likely that – as in the Jiangsu-to-Taiwan case – there will eventually be laboratory-confirmed cases in the EU who have acquired the infection in China or other yet unrecognised affected areas. It is important to acknowledge that such cases would not indicate a change in risk, although a laboratory-confirmed case in the EU would be a major communication event. Equally, Europe will need to prepare for this eventuality in terms of surveillance, infection control, clinical care, and investigation. The period

when a person with A(H7N9) infection is infectious is not known. However, patients admitted to hospital with A(H7N9) infection are likely to be viraemic and to excrete the virus in body fluids and therefore pose an infection risk to staff and other patients [63]. The risk of transmission to humans taking place in Europe through any route must be considered extremely low at present. Specifically, there is no epidemiological evidence to date that avian influenza can be transmitted to humans through consumption of food, notably poultry meat and eggs.

There is no specific guidance on blood or tissue donor deferral for exposure to avian influenza. The incubation period for A(H7N9) is assumed to be 10 days or less, and there is no reason to believe that infected people will be viraemic beyond the acute disease episode [10]. Therefore, the risk of transmission through blood transfusion can be considered very low in the context of the current donor selection procedures.

There is insufficient evidence to quantify the risk of A(H7N9) developing into a virus that transmits from human to human. Close monitoring of the outbreak epidemiology, clinical features and the viruses' genetic variation over time will be critical for assessing this risk; instruments like the Influenza Risk Assessment tool can be used for this task [61, 62]. However, that risk is certainly not zero. This emphasises the importance of European countries continuing to prepare for the eventuality of future pandemics, including the precautionary development of human vaccines and increased monitoring of animal influenzas at the animal/human interface [63-66].

The risk of A(H7N9) virus being transported to Europe in viraemic poultry through legal trade is negligible. EU regulations do not permit the importation of live poultry, their day-old chicks and hatching eggs and birds other than poultry (captive birds such as parrots, finches and ornamental birds) from China. The only poultry commodities authorised for import from China into the EU are sterilised meat products (all provinces), heat-treated poultry meat (only from Shandong Province), and egg products that have been heat treated. Given the very heat-labile nature of all influenza viruses, these commodities are not considered to pose any risk of any influenza virus transmission to consumers.

The risk of the A(H7N9) virus arriving in Europe with migratory birds cannot be quantified at this time. ECDC and the European Food Safety Authority (EFSA) have performed multiple independent risk assessments in the past regarding avian influenza that would to a large extent also cover pathways for A(H7N9) [61, 67, 68,69-71]. The hypothesis that poultry in the affected area have been infected by wild birds has not been confirmed. Surveillance in wild birds for this novel virus has yet to be undertaken in any country. Also, the geographic distribution of infected poultry remains to be established. Although A(H5N1) viruses were detected in wild bird populations in Asia as early as 1996, it took nearly a decade for them to be identified in wild birds in Europe. While there are some short-term threats to Europe from these viruses, the approach that ECDC now recommends concerning surveillance is more medium and long-term and should generalise across all animal influenzas, not just A(H7N9).

Anticipated technical developments in the immediate future

- Regular coordinated epidemiological updates by WHO, ECDC, the Chinese CDC, FAO, etc. (initially on a weekly basis).
- Regular dissemination of information to stakeholders from ECDC through its website, social media, and distribution lists for the ECDC Flu and Other Respiratory Viruses Digest.
- A survey of Member States by ECDC, CNRL and the WHO Regional Office for Europe of the methods used at national influenza centres for the detection and diagnosis of A(H7N9) infection.
- Identification of triggers through ECDC that would indicate a need for even more accelerated action in Europe, e.g. the detection of human-to-human transmission in Europe.
- ECDC review/update of certain parts of its guidance on other avian influenzas so that it is suitable also for A(H7N9).
- The results of pathogenicity testing and other animal experiments with the A(H7N9) viruses in OFFLU laboratories (the OIE-FAO global network of expertise on animal influenza).
- The validation of animal tests for A(H7N9) in China and, if necessary, amendments to the test strategy and actual tests. This then may be followed by reappraisal of the burden of infection in birds.
- Further animal sector epidemiology and information concerning the reservoir of infections in China including poultry and wild birds.
- Development of candidate vaccine viruses and associated reagents by the WHO regulatory laboratory.
- Agreement on laboratory serological testing strategies by CONSISE.

Recommendations for the EU/EEA

- EU/EEA citizens working in or visiting China should avoid visiting live bird and animal markets ('wet markets') because of the potential presence of avian influenza viruses that are pathogenic to humans in these markets. They should also avoid direct contact with bird and animal faeces, untreated bird feathers and other animal and bird waste, and they should follow basic hand hygiene rules, e.g. hand-washing with soap and the use of alcohol-based hand rubs.
- Specific food safety recommendations for the EU are not required for the A(H7N9) outbreak. Longstanding advice that chicken and eggs should be properly cooked remains relevant [75, 76].
- As there is no evidence of human-to-human transmission at this stage, tracing contacts of passengers that are symptomatic during a flight from China and chemoprophylaxis for them is not recommended, but could be considered if the case is later confirmed [77].
- National public health authorities and infection control managers should alert and remind clinicians and healthcare workers of standard guidance for investigation, infection control and contact tracing around cases of severe acute respiratory infections [33, 78, 79].
- Public health authorities should investigate all clusters of severe respiratory infections and infections in healthcare workers who have been caring for patients with severe acute respiratory disease, considering a number of pathogens, not just A(H7N9).
- Deferral criteria for blood safety should stay unchanged, i.e. persons with acute febrile illness should not donate blood until after they have fully recovered.
- National public health authorities should make preparations for the occurrence of confirmed cases in the EU/EEA Member States; authorities should consider active tracing, follow-up of close contacts and people who may have shared the same exposure, as well as antiviral prophylaxis following standard guidance and protocols.
- Diagnostic and reference laboratories in the EU should continue to use their current generic RT-PCR assays for influenza A for screening and testing of possible cases if those tests are based on highly conserved internal gene sequences (e.g. in the M gene segment). The match of primers and probes to the published sequences of the A(H7N9) virus should be checked and validated with available positive control material.
- Clinicians and laboratory specialists should be reminded to consider the possibility of animal influenza infection in persons with severe acute respiratory infection who have, in the previous ten days, been in China and other countries with circulating animal influenza viruses pathogenic to humans.
- Influenza A virus isolates that cannot be sub-typed at national reference laboratories must be sent rapidly to the WHO Collaborating Centre for Reference and Research on Influenza based in Europe.
- Member States should not report cases under investigation for A(H7N9) at the EU level before confirmation. However, clinicians and laboratories may need to nottify their national authorities about cases under investigation according to national guidelines.
- Any confirmed case being diagnosed in the EU/EEA area should be immediately reported to international authorities by national contact points through the Early Warning and Response System (EWRS) and to WHO under the International Health Regulations (2005). Reporting through EWRS qualifies as IHR notification and avoids double reporting.
- Some population-based age-specific serological surveys of likely immunity to A(H7N9) should be undertaken using CONSISE protocols or their national equivalents in a number of populations.
- In accordance with WHO guidance, EU/EEA Member States should not implement travel or trade restrictions for countries with A(H7N9) transmission.
- Member States should liaise with their national animal health agencies to ensure timely exchange of surveillance data for A(H7N9) and consideration should be given for how joint 'One Health' surveillance might be undertaken.
- EU/EEA countries should continue to review and update their pandemic preparedness plans, not only for A(H7N9) but for any novel virus; this process should include the lessons learnt from the 2009 pandemic and new guidance from WHO.

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