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AVIAN INFLUENZA, HUMAN (52): H7NX SEQUENCE ANALYSIS

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Outbreak management by the use of influenza A(H7Nx) virus sequence analysis

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Introduction

This article drawing a parallel between an influenza A(H7N7) outbreak in the Netherlands in 2003 and the current influenza A(H7N9) virus infections in China

The recently identified human infections with avian influenza A(H7N9) viruses in China raise important questions regarding possible source and risk to humans. Sequence comparison with an influenza A(H7N7) outbreak in the Netherlands in 2003 and an A(H7N1) epidemic in Italy in 1999-2000 suggests that widespread circulation of A(H7N9) viruses must have occurred in China. The emergence of human adaptation marker
PB2 E627K in human A(H7N9) cases parallels that of the fatal A(H7N7) human case in the Netherlands.

Background

Since 31 Mar 2013, Chinese health authorities have been reporting human cases of avian influenza A(H7N9) virus infections. This novel reassortant influenza virus, carrying 6 internal gene segments of poultry A(H9N2) viruses, supplemented with a haemagglutinin (HA) subtype 7 and a neuraminidase (NA) subtype 9 originating from wild birds [1,2], has caused infections in at least 82 [now 87] persons, of whom 17 have died, as of 17 April 2013. The human infections occurred in eastern China in 4 provinces (Henan, Anhui, Jiangsu, and Zhejiang) and 2 municipalities (Shanghai and Beijing). Currently, the source of the human infections is unclear. However, in response to the detection of the influenza A(H7N9) virus among chickens, pigeons, ducks, and environmental samples from some animal markets, as reported to the World Organisation for Animal Health (OIE), Chinese authorities have suspended live poultry trade and implemented the immediate closure of poultry markets, launched road inspections for transport of poultry, and have culled birds in an effort to deal with the issue. The outbreak raises important questions regarding possible source and risk to humans, and these will be addressed through case investigations.
Here, we compare some findings from the 1st 2 weeks of the outbreak with those from a large highly pathogenic avian influenza (HPAI)
A(H7N7) virus outbreak in the Netherlands in 2003 and from a low pathogenic avian influenza (LPAI) A(H7N1) epidemic in Italy in
1999-2000 [3-5] and discuss issues related to diagnosis and the use of molecular surveillance to monitor the outbreak.

Influenza A(H7N7) outbreak in the Netherlands in 2003

Exactly 10 years ago, the Netherlands was struck by an HPAI A(H7N7) virus outbreak that resulted in the infection of poultry on 255 farms and the subsequent culling of about 30 million chickens. A total of 453 exposed persons had mild symptoms and were investigated, of whom
89 were laboratory-confirmed as having an A(H7N7) virus infection [6,7].

Diagnosis of influenza A(H7Nx) virus infection

[Abbreviated; see original text via the source URL above to view the illustrations, tabulated data, and references]

During the HPAI A(H7N7) virus outbreak in the Netherlands, almost all human cases had mild symptoms, particularly conjunctivitis, but one veterinarian died after an episode of severe influenza-like illness complicated by acute respiratory distress syndrome (ARDS) [7] .
Diagnosis was based on virus detection by reverse transcription polymerase chain reaction (RT-PCR) from eye swabs, or combined nose and throat swabs. An important observation was that the sensitivity of eye swab-based diagnostics was much higher than that of diagnostics based on combined nose and throat swabs [6,7]. Similarly, in later sporadic infections of humans with H7 influenza A viruses, ocular symptoms were observed, probably caused by a preference of H7 influenza viruses for receptors in the eye [8]. Studies have shown that H7 influenza viruses may use the ocular mucosa as portal of entry for systemic infection and that this is strain dependent [9,10]. Such symptoms have not been described for the cases of A(H7N9) virus infection in China in 2013, but it may be important to actively monitor for conjunctivitis in the outbreak investigation, as it may increase the success of case finding, particularly for mild cases.

Serological surveillance is important to rule out infection in patients sampled too late for direct virus detection and to assess the extent of transmission. This may be a problem since serological responses in persons with confirmed influenza A(H7Nx) virus infection have been difficult to detect, making assessment of A(H7N9) virus exposure using serosurveys challenging [11,12]. However, determining the kinetics of the antibody response in confirmed cases of influenza A(H7N9) virus infection will provide important information that can inform public health action.

Sequence analysis of the Dutch viruses detected in poultry and in humans showed rapid diversification of the outbreak strain into multiple lineages (Figure). On the basis of the combined epidemiological and laboratory analyses, we demonstrated that sequences from humans were positioned mostly at ends of the branches of minimal spanning trees, confirming that humans were probably not involved in onward transmission [3].

In the current study, we compared the sequence diversity observed during the Dutch A(H7N7) outbreak and Italian A(H7N1) epidemic with the initial A(H7N9) virus sequences from the current outbreak in China. The maximum genetic distance generated during the 3 months of the Dutch HPAI A(H7N7) outbreak in concatenated HA, NA and PB2 segments of A(H7N7) viruses was 25 nucleotide substitutions. For the Italian LPAI A(H7N1) epidemic, the distance generated during a 9-month period was 66 nucleotide substitutions. For the A(H7N9) outbreak strains, this genetic distance is 35 substitutions, or 21 substitutions when the outlier strain A/Shanghai/1/2013 is ignored (Figure).

All (n=7) NA sequences of the A(H7N9) viruses are characterised by a deletion in the stalk region, associated with adaptation to gallinaceous hosts [1,2,13]. Similar deletions in the NA stalk were also observed during the A(H7N7) outbreak in the Netherlands and the A(H7N1) epidemic in Italy [5]. Given the degree of sequence diversity present in initial A(H7N9) virus sequences, compared with that of the Dutch HPAI A(H7N7) and Italian LPAI A(H7N1) outbreak strains, and the large geographical area affected, the data are suggestive of (silent) spread and adaptation in domestic animals before the novel A(H7N9) virus was identified in humans.

Human adaptation markers

The majority of the Dutch human cases of A(H7N7) virus infection had mild symptoms, with the exception of one fatal case who was diagnosed with an A(H7N7) virus with the mammalian adaptation marker PB2 E627K.
This mutation most probably occurred during infection of this case and was associated with high virulence [14]. Remarkably, the PB2 segments of the 4 available human virus genome sequences from China all carry this E627K substitution, which is absent in the virus isolates obtained from birds and the environment [2]. In addition, 3 of the 4 infections with the virus with PB2 E627K were fatal.
There are 2 plausible explanations for this observation:
- the mammalian adaptation markers are selected during replication in humans following exposure to viruses that do not have this mutation, which are circulating in animals;

- the mammalian adaptation markers result from virus replication in animals from which humans become infected.

The relatively protracted disease course in the current outbreak of A(H7N9) virus infection, with relatively mild symptoms at first, followed by exacerbation in the course of a week or longer, is suggestive of the 1st hypothesis, similar to the outbreak in the Netherlands. In this scenario, an important difference in the A(H7N7) observations from the Netherlands is the frequency of finding the PB2 E627K mutation in humans (4/4 A(H7N9) sequenced patient strains compared with 1/61 sequenced A(H7N7) patient strains). Therefore, an outstanding question is whether the A(H7N9) viruses are more readily mutating in humans or milder cases are being missed. Contact investigations have found no mild cases and only one asymptomatic case), but in order to address this issue, more enhanced testing of persons exposed to a similar source is needed, using the most sensitive tests available on the optimal clinical specimen type obtained at the right time.

Although human infections with H7 influenza viruses have occurred repeatedly over the last decades without evidence of sustained human-to-human transmission, the absence of sustained human-to-human transmission of A(H7N9) viruses does not come with any guarantee. 5 of 7 A(H7N9) virus strains obtained from humans (n=2), birds (n=2), and the environment (n=1) have a mutation in HA, Q226L, that is associated with binding to alpha(2,6)-linked sialic acids, the virus receptors in the human upper respiratory tract [2]. This Q226L substitution in combination with G228S has been associated with human receptor preference for influenza viruses that caused the pandemics of 1957 and 1968 and with airborne transmission of A(H5N1) virus [15,16]. For H7 viruses, it has recently been demonstrated that these mutations also increased human receptor-binding affinity [17]. In combination with the PB2 E627K mutation, the A(H7N9) virus thus contains 2 well-known mammalian adaptation markers.

Conclusion

Comparative analysis of the 1st virological findings from the current outbreak of influenza A(H7N9) virus infection in China with those from other influenza A(H7Nx) outbreaks suggests that widespread circulation must have occurred, resulting in major genetic diversification. Such diversification is of concern, given that several markers associated with increased risk for public health are already present. Enhanced monitoring of avian and mammalian animal reservoirs is of utmost importance as the public health risk of these A(H7N9) viruses may change following limited additional modification.

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[On the basis of an analysis of a large highly pathogenic avian influenza (HPAI) A(H7N7) virus outbreak in the Netherlands in 2003 and a low pathogenic avian influenza (LPAI) A(H7N1) epidemic in Italy in 1999-2000, the authors are arguing that a similar approach will be appropriate in analysis of the evolving epidemic of avian A/(H7N9) virus infection in China. - Mod.CP]

[see also:
Avian influenza, human (51): H7N9 update 20130418.1655610 Avian influenza, human (50): China H7N9 update 20130417.1653194 Avian influenza, human (48): China H7N9 update 20130416.1650582 Avian influenza, human (47): China H7N9 update 20130415.1647864 Avian influenza, human (46): China: H7N9 stealth virus 20130415.1647713
Avian influenza, human (45): China: H7N9, update 20130414.1645270 Avian influenza, human (44): China (HE), H7N9 20130413.1643923 Avian influenza, human (43): China, H7N9 update 20130413.1643270 Avian influenza, human (42): China (BJ), H7N9 20130413.1642086 Avian influenza (35): China, LPAI H7N9, update 20130412.1641185 Avian influenza, human (41): China H7N9 update 20130412.1641464 Avian influenza, human (40): China H7N9 update 20130411.1638767 Avian influenza, human (39): China (SH, JS, ZH) H7N9 update 20130410.1636073

Avian influenza, human (38): China (SH, JS) H7N9 update
20130409.1633860
Avian influenza, human (35): China (SH, JS) H7N9 update
20130408.1630825
Avian influenza, human (34): China (SH, AH) H7N9, RFI
20130407.1628848
Avian influenza, human (33): vaccine development 20130407.1628472 Avian influenza, human (32): China (SH, AH) H7N9 20130407.1628294 Avian influenza, human (31): China (Shanghai) H7N9 20130406.1626812 Avian influenza, human (30): China (Hong Kong, Taiwan) H7N9, NOT
20130406.1626565
Avian influenza, human (29): China (ZH) H7N9, market quail
20130406.16264
Avian influenza, human (28): China H7N9, WHO 20130406.1626360 Avian influenza (28): China (SH) H7N9, OIE, update 20130405.1624901 Avian influenza, human (27): H7N9 update, more fatalities
20130405.1624260
Avian influenza, human (26): China H7N9 case list & map
20130404.1623110
Avian influenza, human (25): China (SH) H7N9, update 20130404.1622647 Avian influenza (27): China (SH) H7N9, avian case 20130404.1621938 Avian influenza (26): China, H7N9, RFI 20130403.0666 Avian influenza, human (24): China (ZJ) H7N9 update 20130404.1621801 Avian influenza, human (22): China (SH) H7N9, fatal: correction
20130404.1621799
Avian influenza, human (22): China (SH) H7N9 fatal 20130404.1621700 Avian influenza, human (20): China (JS) H7N9 patient details
20130403.1617279
Avian influenza, human (16): China (SH, AH) H7N9 WHO 20130401.1614707]