Background: A(H7N9) avian influenza virus has caused at least 132 human cases and 37 deaths in China since its emergence in February/March 2013. This has led to significant concerns that it could in the future spread globally, causing the next influenza pandemic. Rapid testing and diagnosis of possible human influenza A(H7N9) virus infections are important diagnostic and public health tasks. An accurate diagnosis will allow the timely administration of antiviral therapy and may also enable quarantine of infected cases to prevent further spread of the virus. Point-of-care tests (POCTs) based on antigen detection are simple to use and are designed for use in a medical clinic or outpatient settings, enabling the rapid testing of patient specimens within 15 minutes. For public health purposes, it is important to determine whether POCTs can detect the novel influenza A(H7N9) virus, particularly as previous studies have found that some POCTs had poorer sensitivity in detecting avian influenza strains compared with circulating human seasonal influenza strains.
Materials and Methods: We evaluated six widely available POCTs (SD Bioline, BD Directigen EZ, BD Veritor, Binax Now, Clearview and Quidel Sofia) for their ability to detect the avian influenza A(H7N9) virus A/Anhui/01/2013, compared with three other recent low pathogenic avian influenza A(H7) viruses and two A(H3N2) and two A(H1N1)pdm09 human seasonal influenza viruses. All viruses were cultured in Madin-Darby Canine Kidney cells, standardised to an infectivity titre of 1 x 10^6 tissue culture infectious dose$_{50}$/mL ($TCID_{50}$/mL) and then diluted in phosphate-buffered saline in half-log_{10} dilutions. Real-time RT-PCR analysis was conducted on each virus dilution to determine a cycle threshold (Ct) value and RNA copy number. Each virus dilution was then tested in each POCT according to the manufacturer's instructions and a limit of detection (LOD), based on either the $TCID_{50}$/mL or the RNA copy number/µL, was determined. Four of the kits were read by eye, while two POCTs (Veritor and Sofia) utilised a mechanical reader. Results: Based on the $TCID_{50}$/mL, the LOD of five of the six POCTs for the A/Anhui/01/2013 influenza A(H7N9) virus ranged from 1 x 10^5 to 1 x $10^{5.5}$ $TCID_{50}$/mL, with the Sofia and Directigen EZ detecting virus at the lower limit. The Clearview POCT was unable to detect the A(H7N9) virus at any of the concentrations tested (1 x 10^6 $TCID_{50}$/mL or lower). In comparison, the LOD of the POCTs for the other influenza A(H7) and seasonal viruses tested was generally better than that seen with the A/Anhui/01/2013 virus. Comparison of POCT LODs based on RNA copy number/µL showed similar results to those based on $TCID_{50}$/mL for four of the kits (Binax Now, Clearview, Veritor and Sofia). These POCTs were less sensitive for the detection of the influenza A(H7N9) virus compared with the seasonal or other influenza A(H7) viruses. However, for the SD Bioline and the Directigen EZ tests, comparison of the LODs based on RNA copy number/µL showed that A(H7N9) was detected at a similar sensitivity to the other viruses. LODs based on RNA copy number/µL or Ct value allowed an estimate of the expected performance of the POCTs in detecting A(H7N9) virus in clinical samples. Comparison of the published matrix gene Ct values of clinical samples from patients with confirmed A(H7N9) infection suggested that five of the six POCTS would have detected only one of the four A(H7N9)-positive clinical specimens, with the other three specimens being outside the LOD of these assays. Conclusions: This study demonstrates that the sensitivity of at least four of the six evaluated POCTs is lower for the novel influenza A(H7N9) virus than for seasonal influenza viruses and the other avian influenza A(H7) viruses tested. Comparison with published Ct values for clinical specimens from A(H7N9) patients suggested that these POCTs may not detect the majority of A(H7N9) cases, particularly if samples are taken late in the course of disease. Therefore, real-time PCR remains the diagnostic test of choice for the testing of suspected A(H7N9) influenza cases.

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The emergence of the 2013 H7N9 and related viruses in China

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Background: The novel H7N9 influenza A virus first detected in March 2013 has caused more than 130 cases of human infection in China, resulting in 39 deaths. This virus is a reassortant of H7, N9 and H9N2 avian influenza viruses and carries some amino acids linked to mammalian receptor

binding, raising concerns of a new pandemic. However, neither the source populations of the H7N9 outbreak lineage nor the conditions for its genesis are fully understood. Materials and Methods: Following the initial reports of H7N9 influenza infection in humans, field surveillance was conducted during 4th-18th April in Zhejiang, Shandong and Guangdong provinces. Pairs of oropharyngeal and cloacal samples from chickens and other poultry, together with faecal and water samples from live poultry markets (LPMs), farms and wetlands were collected for virus isolation and whole genomic sequencing. H7, N9, N7 and H9N2 archived isolates, obtained during previous influenza surveillance between 2000-2013 in southern China, were also sequenced and phylogenetically analyzed to pinpoint the genesis of the H7N9 and a related H7N7 virus. The infectivity and pathology of H7N9 and H7N7 viruses were tested in a ferret model. Results: Through a combination of active surveillance, screening of virus archives, and evolutionary analyses, we found that H7 viruses have independently transferred from domestic ducks to chickens in China on at least two occasions. Subsequently they reassorted with enzootic H9N2 viruses to generate the H7N9 outbreak lineage, and a related but previously unrecognized H7N7 lineage. The H7N9 outbreak lineage has spread over a large geographic region and is prevalent in chickens at LPMs that appear to be the immediate source of human infections. In ferrets this virus caused a productive infection and pneumonia. Virus was shed via the nasal route and transmitted to physical contact and some airborne-exposed animals. Like the H7N9 virus, the H7N7 virus was also mainly isolated from chickens at LPMs and it could efficiently infect ferrets, be shed via the nasal and rectal routes, and cause severe pneumonia. Conclusions: These findings provide a clear picture showing how the current H7N9 human viruses emerged. Domestic ducks act as primary vectors to acquire and maintain diversified viruses from migratory birds, and facilitate different subtype combinations between H7 and N9 or N7 viruses and interspecies transmissions to chickens. After being introduced, the H7N9 or H7N7 viruses reassorted with enzootic H9N2 viruses and formed the current reassortant H7N9 or H7N7 viruses seen in chickens. This likely led to outbreaks in chickens, resulting in the rapid spread of the novel reassortant H7N9 virus through LPMs, which then became the source of human infections. Whether the H7N9 outbreak lineage will, or has, become enzootic in China needs further investigation. Our results also indicate that H7 viruses pose a broader threat than the current H7N9 virus. Continued prevalence of this family of H7 viruses in poultry could lead to further sporadic human infections, with an ongoing risk that the virus might acquire efficient human-to-human transmissibility.

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Virological characteristics and serological response of the human case of influenza due to novel avian-origin reassortant H7N9 virus in Taiwan

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Background: On 24 April 2013, the first imported case of H7N9 infection was confirmed in Taiwan in a patient who returned from China on 9 April. We described the clinical course and viral dynamics in the airway and blood, and the viruses isolated from the airway were studied for resistance mutations to neuramindase inhibitors and receptor specificity. Materials and Methods: Serial respiratory and blood specimens were collected from the case patient to determine the H7N9 viral load with the use of real-time RT-PCR, and the antibody titers in serial blood specimens were determined with the use of hemagglutination inhibition assay. The viruses isolated from the respiratory specimens were characterized for their ability to replicate in different cell lines and binding ability to α-2,3-linked orα-2,6-linked sialic acid; and sequencing of the virus genes was performed. Results: Despite treatment with oseltamivir that was begun at a daily dose of 150 mg on 17 April, the patient developed progressive pneumonia with respiratory failure. A more than 1-log reduction of viral load in the sputum specimen was detected 5 days after the change of oseltamivir daily dose to 300 mg, when the viral