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Ongoing transmission of avian influenza A viruses in Hong Kong despite very comprehensive poultry control measures: A prospective seroepidemiology study

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Summary *Objectives:* Stringent measures have been implemented in Hong Kong to prevent human infections due to avian influenza viruses (AIVs). Here, we report the seroprevalence of AIVs among high risk population.
Methods: In this prospective study, blood samples were collected in October and November 2013 and in July 2014 from workers at live poultry market (LPM) and pig/cattle slaughterhouse (SH) in Hong Kong. Serum antibody titers against A(H5N1), A(H7N9) and A(H9N2) were determined.
Results: When an hemagglutination inhibition (HI) titer of 40 was used as the cutoff, the A(H5N1) seropositive rate among LPM workers increased from 0% in 2013 to 37.8% in 2014 ($P < 0.001$) and the A(H9N2) seropositive rate increased from 10% to 55.6% ($P < 0.001$). There was no significant increase in A(H7N9) seropositive rate for LPM workers irrespective of cutoff titer. For SH workers, there was no significant increase in HI titer for any AIVs. Significantly more LPM workers had a $\geq$4-fold increase in A(H5N1) HI titer from 2013 to 2014 than SH workers (60% vs 8.3%, $P = 0.020$).

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Conclusions: There was a significant increase of serum A(H5N1) and A(H9N2) HI titers among Hong Kong LPM workers between 2013 and 2014. Although we cannot exclude some degree of antibody cross-reactivity with other influenza viruses, our results suggest the occurrence of subclinical AIV infections in this population.

Introduction

Avian influenza viruses (AIVs) have been known to cause human disease since the 1950s.[1] Before 1997, only sporadic human infections caused by AIVs were reported, and most patients had mild upper respiratory tract symptoms or conjunctivitis without serious complications. In 1997, 18 patients were diagnosed to have A(H5N1) virus infection in Hong Kong with 6 deaths, and the outbreak was only stopped after territory-wide culling of live chickens.[2] Human infections with A(H5N1) virus has been subsequently reported from other parts of Asia, Europe, Africa and North America.[3,4] In 2013, human infections with A(H7N9) virus were first reported from the Yangtze River delta in eastern China,[5–7] and imported cases have been reported from Hong Kong, Taiwan, Malaysia and Canada.[8–11] With case-fatality rates of 53% and 38%, A(H5N1) and A(H7N9) viruses are currently two of the most virulent respiratory viruses that cause human outbreaks.[11,12]

In addition to A(H5N1) and A(H7N9) viruses, Hong Kong is also under constant threats from other AIVs. In 1999, A(H9N2) virus was first reported to cause human infections in Hong Kong,[13] and human cases have been subsequently reported in Hong Kong, mainland China, Bangladesh, and Egypt.[14–17] A(H5N6) and A(H10N8) viruses have caused sporadic fatal human cases in mainland China,[1,18] and A(H6N1) virus has infected a person in Taiwan.[19]

The Hong Kong government has implemented numerous measures to prevent human infections with AIVs.[7] All live chickens must be supplied by registered farms and must be vaccinated against A(H5N1) virus. Each batch of live chickens is tested for A(H5N1) and A(H7N9) viruses. AIV testing is performed for all carcasses of dead wild birds. AIV surveillance is conducted regularly at live poultry markets (LPM), pet bird shops, recreational parks and nature reserves. Overnight storage of live poultries in LPM is banned. When A(H5N1) or A(H7N9) viruses are found in poultries, affected live poultries are culled and selling of live poultries are banned for 21 days. With these preventive measures which are amongst the most stringent in the world, only sporadic local cases of human infections with A(H9N2) virus and no local cases of human infections with A(H5N1) or A(H7N9) viruses have been reported after 1997.[14]

Previous seroprevalence studies suggested that subclinical human infections with influenza viruses are common.[20–24] To assess whether subclinical human infections due to AIVs occur in Hong Kong, we compared the seroprevalence of AIVs in LPM workers in 2013 and 2014. As a control, we also assessed the seroprevalence of AIVs among slaughterhouse (SH) workers.

Materials and methods

Study design

In this prospective study, blood samples were collected in October/November 2013 and July 2014 from workers at LPM and SH in Hong Kong. LPM workers were staff members working in poultry wholesale markets or poultry retail markets, while SH workers were staff members working in pig or cattle SH. All LPM or SH workers in Hong Kong were invited. Written informed consents were obtained from all participants who volunteered to be enrolled in this study. Individuals were excluded if they refused to provide consent. Demographic data, underlying diseases, exposure history, and influenza vaccination history were obtained using a standardized questionnaire. Underlying diseases were classified according to the risk factors for severe disease published by the World Health Organization.[25] Data were entered into a predesigned database. Seroprevalence was assessed using hemagglutination inhibition (HI) assay.[22,23] This study has been approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

Blood collection, transport, processing and storage

For each study participant, 10 ml of blood were collected into VACUTTE® Z Serum Clot Activator blood collection tubes. The specimens were transported to the laboratory within 4 h of blood collection. After arriving at the laboratory, the blood specimens were centrifuged at 2000 rpm for 10 min. The sera were collected and stored at −80 °C until hemagglutination inhibition antibody titer testing.

Hemagglutination inhibition (HI) assay

HI assays for A(H5N1), A(H7N9) and A(H9N2) viruses were performed as described previously.[22,26–28] Briefly, HI assay was performed by standard microtiter techniques after removal of non-specific inhibitors in serum by adsorption with turkey or horse erythrocyte and then with receptor destroying enzyme (RDE) (1:3), incubation overnight at 37 °C and followed by heat-inactivation at 56 °C for 30 min. Serial 2-fold dilutions of RDE-treated serum from 1:10 were titrated against 4 hemagglutinin units of reference antigens using 0.5% turkey erythrocyte or 1% horse erythrocyte. Virus strains used were A/Vietnam/1194/2004 (H5N1), A/Anhui/1/2013 (H7N9) and A/Hong Kong/1073/99 (H9N2).[29–31] Turkey erythrocyte was used in the HI assay for all 3 AIVs, while horse erythrocyte was also used in the HI assay for A(H7N9) virus as recommended by the World Health Organization.[27] All serum samples from each subject were tested