Conclusions: The lack of substantial change in preventive measures or knowledge about the modes of H1N1 transmission in the general population suggests that community mitigation measures played little role in mitigating the impact of the first wave of 2009 influenza A(H1N1) pandemic in Hong Kong.

Ref. No.: PHE-1&10 (HKU)

P84-Ab0047

Behavioural Changes in Relation to Risk Perception and Prevention of Avian and Human Influenza in the General Population of Hong Kong, 2006 to 2010

Qiuyan Liao, Benjamin Cowling, Wendy WT Lam, Richard Fielding
The University of Hong Kong, Hong Kong

Background: The Hong Kong government has introduced a series of progressive measures on importation, farming and retail of live poultry to minimize risk of A/H5N1 transmission since 1997. Perceived risk of A/H5N1 and related preventions could decline as these macro-level policies minimizing human-chicken contact. This may paradoxically increase population risk of other influenza and respiratory infection due to reduced preventive behaviors.

Objectives: A follow-up survey in 2010 was conducted to investigate change of live poultry exposure, risk perception and prevention of A/H5N1 among respondents who participated in the random household telephone survey in 2006.

Methods: Totally, of 1,760 respondents who completed the 2006 survey, 680 could be traced and 461 (68%, 461/680) agreed and completed the repeated telephone survey between July and August 2010. Prevalence of buying and touching, perceived risk of A/H5N1, worry, and protective hygiene practices were compared between 2006 and 2010 using descriptive analysis. How changes of these variables differed by respondents' demographics and change of perceived risk and worry leaded to change of buying and practices of hygiene were further explored by multivariate logistic regression analyses.

Results: Prevalence of household buying live poultry declined from 73% in 2006 to 41% in 2010. Buying household bought on averaged 11.4 chicken/household/year in 2010 versus 14.4 in 2006 while touch rate remained unchanged (5%). Overall exposure (touch rate × purchase rate) declined by 21% from 2006 to 2010 (0.72 vs. 0.57 exposure/household/year). Most personal hygiene practices improved from 2006 to 2010 except that frequency of daily hand-washing and covering mouth when sneezing and coughing declined. Male respondents reported less likely to cover mouth when sneezing or coughing (OR=1.60, 95%CI: 1.00-2.56) while immigrants were more likely to report reduced frequency of daily hand-washing (OR=1.58, 95%CI: 1.04-2.41). Perceived risk from buying live poultry and worry about contacting A/H5N1 declined from 2006 to 2010. Younger respondents were more likely to report declined worry and risk from buying live poultry. Declined worry was associated with less hygiene practices (OR=1.61, 95%CI: 1.04-2.47).

Conclusions: The decline in buying prevalence may be attributed to limiting poultry availability. However, among buyers, prevalence of touching poultry remained unchanged, suggesting little effect from public health promotion to change purchasing-related behaviours. Perceived risk from buying, A/H5N1 worry and some hygiene practices declined, suggesting that risk of contracting human influenza viruses could increase. Young males and immigrants should be the major target for public health education to promote hygiene practices.

Ref. No.: 09080732

P85-Ab0050

Mass Spectrometrical Identification of Host Cell Surface Protein Receptor(s) for Human Norovirus in Primary Human Duodenal Tissues

Martin C.W. Chan
The Chinese University of Hong Kong, Hong Kong

Introduction: Human noroviruses (NoVs), a member of the family Caliciviridae in the genus Norovirus, is the leading cause of acute non-bacterial gastroenteritis worldwide which affects all age groups in both developed and developing countries. Our understanding on the pathogenesis of NoV has been severely hampered by the lack of a robust in vitro cell culture system and small animal model for NoV. We believe identifying candidate host cellular receptor(s)/co-receptor(s) for NoV may provide important data to the direction of developing an in vitro cell culture system. Laboratory investigations of natural NoV infections and volunteer challenge studies have demonstrated NoV-associated histopathological changes in human small intestines. However, direct evidence showing viral antigens in infected intestinal epithelial cells has been lacking. This raises the concern whether NoV infects enterocytes.

Methods: In this study, we first used in vitro whole-virus binding assay to study NoV tissue tropism. Total protein lysate of human duodenal biopsy specimens were then subjected to virus overlay protein binding assay (VOPBA) and mass spectrometry to identify candidate host cellular receptor(s)/co-receptor(s).

Results:

1. Using in vitro whole-virus binding assay, human norovirus genogroup II genotype 4 (NoV GII.4) Sakai strain was found to bind to human duodenal lamina propria and Brunner's glands.
2. Using virus overlay protein binding assay (VOPBA) and mass spectrometry on total protein lysate of human duodenal biopsy specimens, nucleolar protein 8 (NOL8) was identified as a candidate host cellular protein receptor/co-receptor for NoV GII.4 Sakai strain.
3. NoV may target intestinal non-epithelial cells.

Conclusions: We provide evidence that NoV GII.4 Sakai strain showed in vitro binding pattern to duodenal lamina propria and Brunner's glands, but not to epithelial enterocytes. NoV may target intestinal non-epithelial cells. NOL8 may be a candidate cellular receptor/co-receptor for NoV and deserves further investigations.

Ref. No.: CU-09-02-05

P86-Ab0052

Identification of Hepatitis B Virus DNA Polymerase Sequences to Predict Virological Response to Entecavir Therapy

Danny Wong[1], Malgorzata Kopaniszen[1], Katsumi Omagari[2], Yasuhito Tanaka[2], Daniel Fong[1], Wai-Kay Seto[1], James Fung[1], Fung-Yu Huang[1], An-ye Zhang[1], Ivan Hung[1], Ching-Lung Lai[1], Man-Fung Yuen[1]
[1]The University of Hong Kong, Hong Kong, [2]Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Background and Aims: Entecavir is a potent antiviral agent that often reduces hepatitis B virus (HBV) DNA to an undetectable level after one year of treatment, but HBV DNA may remain detectable in some patients. We aimed to determine whether baseline HBV reverse transcriptase (rt) sequence polymorphism

and quasispecies complexity and diversity were associated with treatment response.

Methods: Pre-treatment HBV DNA levels, HBV rt sequence, and serology from a cohort of 305 entecavir-treated patients were determined. Quasispecies complexity and diversity were determined by clonal sequencing and analyzed using MEGA software. These data were tested for the association with year one virological outcome, defined by optimal response (undetectable HBV DNA; ≤12 IU/mL) or partial response (detectable HBV DNA).

Results: Four rt variants were more frequently detected in the 64 partial responders than in the 241 optimal responders (43.8-51.6% vs. 20.1-27.4%, all P < .05). Multivariate analysis revealed that high baseline HBV DNA (P < .0001; odds ratio [OR] = 2.31), hepatitis B e antigen (HBeAg)-positivity (P < .001; OR = 3.68) and rt124N (P = .001; OR = 3.09) were associated with partial entecavir response. Molecular docking model suggested that rt124N possibly cause a slight steric hindrance to entecavir binding. Compared with the optimal responders, the partial responders had a lower quasispecies complexity and diversity at both the nucleotide and amino acid levels.

Conclusions: Apart from the known factors (high baseline HBV DNA, HBeAg-positivity), a novel single nucleotide polymorphism (rt124N) and lower quasispecies complexity and diversity were associated with partial entecavir response at year 1. Identification of these viral factors can assist in choosing an optimal antiviral treatment for individual patients.

Ref. No.: 08070852

P87-Ab0053
Application of Co-amplification at Lower Denaturation Temperature-PCR (COLD-PCR) Sequencing for the Early Detection of HBV Antiviral Drug Resistant Mutations

Danny Wong, Ottilia Tsoi, Fung-Yu Huang, Wai-Kay Seto, James Fung, Ching-Lung Lai, Man-Fung Yuen
The University of Hong Kong, Hong Kong

Background and Aims: Nucleoside/nucleotide analogue for the treatment of chronic hepatitis B virus (HBV) infection is hampered by the emergence of drug resistance mutations. Conventional PCR-sequencing cannot detect minor variants of <20%. We aimed to develop a modified CO-amplification at Lower Denaturation temperature-PCR (COLD-PCR) method for the detection of HBV minority drug resistance mutations. During thermal-cycle of COLD-PCR, the denaturation temperature (normally at 95°C) was reduced to a lower-than-normal critical denaturation temprature. Using a critical denaturation temperature, minor variants can be enriched during PCR.

Methods: The critical denaturation temperature for COLD-PCR was determined using real-time PCR. Sensitivity of COLD-PCR sequencing was determined using serially-diluted plasmids containing mixed proportions of HBV reverse transcriptase (rt) wild-type and mutant sequences. The performance of COLD-PCR sequencing was compared to that of conventional PCR-sequencing and a line probe (LiPA) assay, using 215 samples obtained from 136 lamivudine- or telbivudine-treated patients with virological breakthrough.

Results: The critical denaturation temperature for COLD-PCR was 78°C. With serially-diluted mixture of wild-type and rt mutant plasmids as templates, conventional PCR-sequencing detected mutations only if they existed in ≥25%, whereas COLD-PCR sequencing detected mutations when they existed in 5-10% of the viral population. Among the 215 clinical samples obtained from the lamivudine- or telbivudine-treated patients, drug resistance mutations were detected in 155 (72 %), 148 (69 %) and 113 samples (53 %) by LiPA, COLD-PCR, and conventional PCR-sequencing, respectively. Nineteen (9 %) samples had mutations detectable by COLD-PCR but not LiPA, while 26 (12 %) samples had mutations detectable by LiPA but not COLD-PCR, indicating both methods were comparable (P = 0.371). COLD-PCR was more sensitive than conventional PCR-sequencing: 35 (16 %) samples had mutations detectable by COLD-PCR but not conventional PCR-sequencing, while none had mutations detected by conventional PCR-sequencing but not COLD-PCR (P < 0.0001).

Conclusions: COLD-PCR sequencing is a simple method which is comparable to LiPA and superior to conventional PCR-sequencing in detecting minor lamivudine/telbivudine resistance mutations.

Ref. No.: 09080682

P88-Ab0058
Effect Modifications of Lifestyle Factors on Risk of Mortality Associated with Influenza in an Elderly Cohort

Chit Ming Wong[1], Wai Man Chan[2], Lin Yang[1], King Pan Chan[1], Hak Kan Lai[1], Thuan Quoc Thach[1], Lai Ming Ho[1], Tai Hing Lam[1], JS Malik Peiris[1]
[1]School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, [2]Department of Health, The Government of the Hong Kong Special Administrative Region, Hong Kong

Background: Influenza has been associated with a heavy burden of mortality and morbidity. The prevention strategy relies on vaccines and antiviral drugs, while there have been few studies which tackled the effects of healthy lifestyle on influenza to provide evidence for promoting an adjunctive approach.

Aims and Objectives: To assess the effects of smoking, alcohol drinking and exercise on influenza associated mortality risks and address the role of health lifestyle as adjuncts to vaccination and antiviral medication in prevention of influenza.

Methods: We collected the baseline lifestyle information of 66,820 persons aged 65 or above recruited by18 Elderly Health Centers (sample fractions 6.5 - 17.2%) in Hong Kong from May 1998 to December 2001. The subjects were followed up for health outcomes till December 2009, by anonymously linking the death records of deceased subjects to the baseline data. Effect modification of each lifestyle factor on influenza associated excess mortality risk from all-natural and cardiorespiratory causes was assessed by time-dependent Cox proportional hazard model. The mortality excess risks associated with influenza for categories of each lifestyle factor were also separately assessed by stratified analysis.

Results: We found that influenza associated mortality risks were higher in ex- and current-smokers compared to never smokers, and the associations were particularly high in female smokers (Wong et al. 2013). Compared to never-drinkers, ex-drinkers had a higher while social/regular drinkers had a lower excess risk associated with influenza. Among the exercise groups, sedentary people had a higher and frequent exercisers had a lower excess risk associated with influenza. An U-shape pattern across the underweight, normal, overweight, moderate obesity and severe obesity groups was observed in influenza associated mortality risks (Yang et al. 2012).