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研究成果報告

香港大學委任研究

Antibiotic Resistance
抗生素抗藥性

Respiratory Infectious Diseases
呼吸道感染疾病

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infection by residential addresses of Hong Kong. There was clear clustering of cases in certain districts of the Kowloon peninsula (such as Kwun Tong) and the New Territories (including Shatin and Tai Po), but Hong Kong Island was relatively spared.

Cluster analysis generated statistical surfaces using the kernel density method to account for SARS incidents on the date of symptom onset and a 5-day incubation period. Each of the time-series kernel maps showed SARS infection rate per 1000 inhabitants on a prototypical day during the 16-week epidemic, with darker zones emphasising disease hot spots.

Contextual analysis enabled construction of daily histograms of the number of observations by 15 classes of infection rates, which were primarily composed of inverse J-shaped curves, showing an increased concentration of SARS occurrences toward the end of March 2003. Origin-and-destination plots of disease clusters were used to explore likely or probable locations of index cases or environmental sources of infection as informed through contact tracing by public health authorities.

Discussion

The kernel method of portraying infection rates provided a means of highlighting locations of disease risks. The related R-values and Moran's coefficients enhanced the analytical context of the point-pattern distributions. Such geospatial intelligence provided the basis for formulating our transmission dynamics model.[2] A variety of approaches ranging from a simple deterministic compartmental approach to a spatially explicit and individual-based simulation were possible in constructing the transmission dynamics model. We based our analyses on a stochastic meta-population compartmental model because the incidence of SARS varied substantially according to geographical districts.

The daily animated series of kernel maps clearly show that SARS was a highly localised disease. In contrast with influenza and measles transmitted through casual contact, the route of transmission for SARS was more compatible with close contact via heavy respiratory droplets and fomites. An alternative interpretation of the observed high degree of geospatial clustering shows that SARS was attributed to an environmental point source outbreak, as demonstrated by faulty sewage systems and the chimney effect hypothesised for the Amoy Gardens SSE. Although it is difficult to gauge retrospectively, had the GIS been available for near real-time analysis, it might have afforded more rapid contact tracing and public health interventions to prevent further large-scale environmental point source outbreaks.

Contextual analysis is a useful adjunct to the usual bio-mathematical modelling approach using reproductive numbers at different points in time throughout the SARS epidemic.[2] The origin-and-destination analysis complemented with R and Moran's I values suggested the direction of spread in a disease cluster that could be used to inform contact tracing and the design of quarantine measures. Instead of isolating entire residential districts as practised in China at the height of the SARS outbreak, these analytical approaches might have enabled better selection of such districts for quarantine.

There are limitations to the GIS technique in infectious disease epidemiology and outbreak investigation. Mapping of diseases tends to expose the 'where' but not 'why there' of the outbreak although map patterns can provide stimuli for generating hypotheses of disease causation. Moreover, newer developments that complement traditional mapping functions such as cluster and contextual analyses can be useful adjunctive investigational tools in outbreak control.

The completeness and rapid availability of necessary data is another area of concern. Conventional field epidemiological data rarely contain the full range of variables required in a GIS analysis. Unfortunately, non-standardisation of patient address formats and missing details diminish the proportion of useable cases for analyses. A number of generic problems associated with information system development must be resolved to render real-time disease monitoring and surveillance. On top of the list is standardisation of data capture documents, as well as procedures and protocols for information management. There is also an urgent need to manage delays in transferring and updating disease information to facilitate rapid analysis and audit of databases. The SARS epidemic is a clear signal that Hong Kong needs much greater investment in health informatics (ie public health information systems, the skills to use them, and networks to share them).

Conclusions

Integration of GIS technology into routine field epidemiological surveillance offers a scientifically rigorous and quantitative method for the identification of unusual disease patterns in real time. Its potential can be synergistically maximised when linked to clinical databases collecting data at the point of care. This integration should entail the whole population and environmental data sources (including meteorological, transportation, topographical information) so as to rapidly recognise, locate and monitor disease outbreaks.

Acknowledgements

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Lai PC, Wong CM, Hedley AJ, et al. Understanding the spatial clustering of severe acute respiratory syndrome (SARS) in Hong Kong. Environ Health Perspect 2004;112:1550-6.

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Viral loads in clinical specimens and SARS manifestations

Key Messages

1. A high viral load in nasopharyngeal aspirate (with or without a high viral load in serum) is a useful prognostic indicator of respiratory failure or mortality. The presence of viral RNA in multiple body sites is also indicative of poor prognosis.
2. Early treatment with an effective antiviral agent before day 10 may decrease the peak viral load, and thus ameliorate the clinical symptoms and mortality, and reduce viral shedding and the risk of transmission.

Introduction

The SARS pandemic affected 8098 people with 774 fatalities in 2002-2003.[1] A novel coronavirus was isolated from SARS patients who had specific seroconversion to this virus.[2-4] Animal models using macaque monkeys, ferrets and domestic cats were established. However, no extrapulmonary lesions could be identified in these animals though virus isolation and real-time polymerase chain reaction (RT-PCR) for viral RNA were positive from their pharyngeal secretions, tracheobronchial secretions, urine, rectal swabs or stool, kidney or lung tissues. We reported the use of RT-PCR to detect SARS-CoV RNA from nasopharyngeal aspirate (NPA), throat swab, urine and stool specimens. We also developed RT-qPCR assays using the LightCycler System (Idaho Technology, Idaho Falls [ID], US) to augment the sensitivity of detection. The serial viral load in NPA was used for monitoring the clinical progress and the response to anti-viral therapy, whereas the admission viral load in serum was used as a marker of prognosis. Unlike the animal models, extrapulmonary manifestations such as haematological changes, diarrhoea, and liver derangement were common in SARS patients. In this study, we assayed and analysed the viral load of clinical specimens from different anatomic sites between days 10 to 15 after the onset of symptoms to understand the role of this virus in the pathogenesis of the clinical manifestations and abnormal laboratory tests in SARS patients.

Aims and objectives

To correlate SARS-CoV viral load in different clinical specimens with the clinical manifestations of SARS.

Methods

Patients who fulfilled the modified World Health Organization definition of SARS (n=154), managed in the United Christian Hospital and Caritas Medical Centre were included in this quantitative virological study. All patients were either serologically confirmed by demonstrating a four-fold rise of indirect immunofluoresent antibody titre against SARS-CoV in the serum taken on admission and within day 28 after symptoms onset, or had positive RT-PCR for SARS-CoV RNA confirmed from their clinical specimens (for those who died or failed to seroconvert before day 28). The case definition included fever of 38ºC or higher, cough or shortness of breath, and new pulmonary infiltrates on chest radiography or high-resolution computed tomography in the absence of an alternative diagnosis to explain the clinical presentation. During the first 15 days, patients were prospectively monitored for occurrence of diarrhoea, oxygen desaturation, mechanical ventilation, and laboratory evidence of lymphopaenia, renal impairment, liver dysfunction, abnormal urinalysis, and mortality. For the diagnosis of SARS-CoV infection, NPA and acute sera were taken on admission. Convalescent sera were taken between days 7 and 28 after the onset of symptoms. In all patients, RT-PCR for SARS-CoV was performed on the NPA collected on admission. RT-qPCR was performed for patients who had their NPA, sera, stool and urine specimens collected on days 10 to 15 after the onset of symptoms. All virological diagnostic laboratory tests including viral culture, RT-PCR, RT-qPCR and immunofluorescent antibody detection for IgG seroconversion against SARS-

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CoV were performed according to our protocols. From clinical samples, RNA was extracted using the QIAamp virus RNA mini kit (Qiagen) as instructed by the manufacturer. For all specimens, 140 μL of the sample were used for RNA extraction and extracted RNA was finally eluted in 30 μL of RNase-free water and stored at -20ºC. For the RT-qPCR assay, RNA and cDNA was generated as described; cDNA was amplified in a 7000 Sequence Detection System (Applied Biosystems) by using TaqMan PCR Core Reagent kit (Applied Biosystems). In a typical reaction, 2 μL of cDNA was amplified in a 25 μL reaction containing 0.625 U AmpliTaq Gold polymerase (Applied Biosystems), 2.5 μL of 10x TaqMan buffer A, 0.2 mM of dNTPs, 5.5 mM of MgCl2, 2.5 U of AmpErase UNG, and 1x primers-probe mixture (Assays by Design, Applied Biosystems). The forward primer was 5'-CAGAACGCTGTAGCTTCAAAAATCT-3' (corresponding to nt 17718 to 17742 of SARS-CoV genome) and the reverse primer was 5'-TCAGAACCCTGTGATGAATCAACAG-3' (complementary to nt 17761 to 17785). The sequence of the reporter probe was 5'-(FAM)TCTGCGTAGGCAA TCC(NFQ)-3' (FAM, 6-carboxyfluorescein; NFQ, non-fluorescent quencher; complementary to nt 17745 to 17760). Reactions were first incubated at 50ºC for 2 min, followed by 95ºC for 10 min. Reactions were then thermal-cycled for 40 cycles (95ºC for 15 sec, 60ºC for 1 min). Plasmids containing the target sequences were used as standard controls. To monitor the integrity of RNA extraction for each sample, a housekeeping gene, beta-actin was detected by RT-PCR using two primers: beta-actin forward, 5'-CCCAAGGCCAACCGCGAGAAGAT-3' and reverse, 5'-GTCCCGGCCAGCCAGGTCCAG-3'. All samples were found to contain detectable beta-actin RNA.

Statistical analysis

All timed data were calculated from the onset of symptoms. We compared the viral load in these specimens with the presence or absence of diarrhoea, oxygen desaturation, mechanical ventilation, lymphopaenia, hepatic dysfunction, abnormal urinalysis and mortality by Chi squared or Fisher's exact test for categorical variables and Mann-Whitney *U* test for continuous variables. A two-tailed P value of less than 0.05 was taken to be significant. Correlation between the number of anatomical sites with detectable viral load by RT-qPCR and mortality was calculated by linear regression.

Results

Viral load in NPA (n=142) between days 10 and 15 after the onset of symptoms was associated with oxygen desaturation (odds ratio [OR]=3.1; 95% confidence interval [CI], 1.6-6.2), mechanical ventilation (OR=11.3; 95% CI, 3.6-35.1), diarrhoea (OR=2.5; 95% CI, 1.3-5), hepatic dysfunction (OR=2.5; 95% CI, 1.2-5.2) and mortality (OR=54; 95% CI, 7-415). Serum viral load (n=53) was associated with oxygen desaturation (OR=5; 95% CI, 1.5-16.4), mechanical ventilation (OR=1.5; 95% CI, 1.1-2) and mortality (OR=17.1; 95% CI, 2.0-151). Stool viral load (n=94) was associated with diarrhoea (OR=14.1; 95% CI, 1.7-114), as was urine viral load (n=111) with abnormal urinalysis (OR=7.2; 95% CI, 1.6-32.9).

Discussion

The viral load reflects the dynamic interaction between viral replication and viral clearance by body defence mechanisms. Viral load study in SARS has been used for virological diagnosis and monitoring of progress or response to anti-viral therapy. In our study, the viral load in the NPA peaked around day 10 and was immediately followed by a decrease with a concomitant normalisation of the lymphocyte count and a corresponding rise of serum antibodies specific for the SARS-CoV. The presence of the virus and the viral load in different body fluids may have a bearing on the possible modes of transmission. The infectivity at day 10 as reflected by a mean peak viral load of 5.8 and 7.0 $\log_{10}$ copies/mL in positive specimens of NPA and stool respectively suggested that respiratory droplets and indirect contact with faeces might be an important mechanism of transmission. Previous viral load study centred on NPA and serum at the time of admission as a diagnostic tool and a prognostic indicator. Viral load study in various body fluids in addition to NPA and serum has not been performed to determine the transmission and pathogenesis of the pulmonary and extra-pulmonary manifestations of SARS.

The SARS is predominantly a viral pneumonia with a rapid tempo of deterioration. The importance of SARS-CoV as a respiratory pathogen is supported by the strong association of the viral load in the NPA with oxygen desaturation, mechanical ventilation and mortality as evident by odds ratios of 3.1, 11.3 and 54 respectively. Unexpectedly it was also associated with diarrhoea (OR=2.5) and hepatic dysfunction (OR=2.5). Anecdotal reports of the usefulness of steroids in the treatment of SARS suggest these extra-pulmonary manifestations could just be part of an inflammatory spill-over from a process of virus induced immuno-dysregulation or excessive cytokine activation in the lungs. However, our findings suggest that viral replication in these extra-pulmonary sites may be as important since the viral load in the stool correlated strongly with diarrhoea. Moreover, electron microscopy of the ileal and colonic biopsy from SARS patients showed numerous viral particles intra- and extra-cellularly.

The serum viral load also correlated with oxygen desaturation, mechanical ventilation, and mortality. This was not surprising, as viraemia has also been reported in adenovirus, respiratory syncytial virus and rotavirus infections.[5-7] However, viraemia even if present is very short lasting in these mucosal infections. In one study, five out of 41 neonates with positive respiratory syncytial virus (RSV) antigen in nasal washes were positive for RSV-RNA in blood. High levels of adenovirus DNA in serum was also associated with fatal outcome in children who developed adenovirus

infection after allogeneic stem-cell transplantation. Six (86%) of seven children who died of adenovirus infection, compared with only two (7%) of 29 other patients, had high serum levels of adenoviral DNA (P<0.0001). The absence of an association between viral load in any specimens with lymphopaenia at day 10 could be explained by the routine use of steroids which induces apoptosis of lymphocytes. The apparent inferior performance of serum viral load as a prognostic indicator could be related to a lower number of available serum samples in this cohort. However, the proportion who had oxygen desaturation in these 53 (38%) patients was not significantly different from the 142 (46%) patients who had submitted nasopharyngeal samples between days 10 and 15.

Compared with other common viral respiratory diseases, the onset of peak viral load in the nasopharynx appeared to be delayed. In a prospective study of viral shedding in nasopharyngeal secretions in experimental adult infections as enumerated by $TCID_{50}$ (median tissue culture infective dose) viral titre or RT-qPCR, RSV was detected between days 2 and 12, with a plateau phase between days 3 and 8 at a peak viral load of 5 $\log_{10}$ copies/mL. In the case of experimental adult influenza, viral replication in NPA peaked at about 48 hours after the onset of symptoms and declined sharply thereafter, with an insignificant degree of viral shedding after days 6 to 8. The peak virus titres in symptomatic volunteers inoculated with influenza A H3N2 ranged from $10^{2.5}$ to $10^{7.0}$ $TCID_{50}$/mL of nasopharyngeal wash. The viral load correlated positively with the clinical symptoms of fever and malaise, as well as the degree of viral shedding. However, the reported low incidence of viraemia and the early peak nasopharyngeal viral load in these two conditions could be accounted by the inherent behaviour of viral replication, background IgG and IgA antibodies with cross-reactivity against homologous antigens (due to previous infections or innate immunity of the host). In many of these experimental infections where the profile of the viral load in NPA was documented, the volunteers were adults and had a low level of background antibodies and therefore concomitant cell mediated immunity against influenza or RSV.

One limitation of the present study was its retrospective nature. Only those who had sent the specimen at around day 10 could be tested and analysed. Changes of lymphocyte subset were also not analysed due to the retrospective nature of this study. Nonetheless, lymphocytes changes in SARS patients were well reported by two other groups who showed a consistent decrease in the peripheral blood level of dendritic cell subsets, natural killer cells, CD4+ and CD8+ T lymphocytes and B lymphocytes in SARS patients.[8,9]

Conclusions

SARS is predominantly a respiratory infection with spread through viraemia to extrapulmonary sites where viral replication leads to non-respiratory manifestations. There could be concomitant immuno-dysregulation and associated inflammatory damage that accentuates its morbidity and mortality. A high viral load in NPA with or without a high viral load in serum is a useful prognostic indicator of respiratory failure or mortality. The presence of viral RNA in multiple body sites is also indicative of a poor prognosis. Early treatment with an effective antiviral agent before day 10 may decrease the peak viral load, and thus ameliorate clinical symptoms and mortality, and reduce viral shedding and the risk of transmission.

Acknowledgements

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Comparative host gene transcription by microarray analysis early after infection of the Huh7 cell line by SARS coronavirus and human coronavirus 229E

Key Message

During the early stages of infection, SARS-CoV produces more severe perturbation of host cell gene expression in a human epithelial cell line of liver origin than the HCoV-229E.

Introduction

SARS-CoV is the aetiological agent of SARS, which is associated with a high mortality and morbidity. Such an unfavourable clinical effect is different from that of other known human coronaviruses, including the group 1 coronavirus 229E and NL63 and the group 2 coronavirus OC43.

SARS-CoV is an enveloped positive-sense single-stranded RNA virus that can grow in embryonic monkey cell lines including the Vero E6 and foetal rhesus monkey kidney (FRhk-4) cells. It can be sub-cultured onto other Vero cells and colonic carcinoma cell lines such as Caco-2 or LoVo. Unlike other human coronaviruses, SARS-CoV proliferates rapidly and causes obvious cytopathic effects in Vero E6 within 48 h of inoculation. There are no other human cell lines known to be susceptible to infection to both SARS-CoV and other human coronaviruses. It has been reported that a human hepatoma cell line (Huh7) can be infected by the pseudotyped lentiviral particles carrying the Spike protein of the SARS-CoV and the wild type replicative SARS-CoV.[1-3]

Aims and objectives

To report the susceptibility of the cell line Huh7 to infection by both the SARS-CoV and HCoV-229E and perform a comparative gene transcriptional profile at an early stage of such infection by these two viruses to elucidate differences in pathogenesis.

Methods

Cell lines and virus

Huh7 cells (courtesy of Prof David Ho, Aaron Diamond AIDS Research Center) were used throughout this study. The cells were incubated at 37ºC in Minimal Essential Medium (MEM) supplemented with 10% foetal calf serum, 100 IU/mL penicillin and 100 μg/mL streptomycin. Our prototype virus (SARS-CoV, HKU-39849) was isolated from the lung-tissue biopsy of the brother-in-law of the index SARS patient who travelled to Hong Kong from Guangzhou and started a superspreading event leading to the pandemic.[4] The HCoV-229E strain (American Type Culture Collection Number: VR-740) was used in this study. The SARS-CoV and HCoV-229E used in our experiments had undergone 3 passages in FRhk-4 cells and MRC-5 cells, respectively, and were stored at -70ºC. Viral titres were determined as the median tissue culture infective dose ($TCID_{50}$) per mL in confluent Huh7 cells in 96-well microtitre plates. The plates were used to standardise the viral inoculum and measure the relative susceptibility of the Huh7 cell line to these two viruses. The relative susceptibilities of Vero 1008, Vero 76, Vero, and Huh7 cell lines to SARS-CoV and HCoV-229E were also tested by $TCID_{50}$. One hundred $TCID_{50}$ was confirmed by plaque assays to be equivalent to 85 plaque-forming units. All work with infectious viruses was performed inside a type II Biosafety Cabinet, in a Biosafety Containment level III facility, and the

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personnel wore powered air-purifying respirators.

Monitoring of virus-induced cytopathic effect, antigen detection, semi-quantitative and quantitative PCR

Huh7 cells and culture supernatants infected with either SARS-CoV or HCoV-229E at a multiplicity of infection of 100 $TCID_{50}$ per cell were collected at 2, 4, 12 and 24 h post-infection. A washing step was performed 1 h post inoculation. The percentages of cells developing cytopathic effects (CPE) were counted by inverted light microscopy at 24 and 48 h. The rate of viral replication was measured semi-quantitatively by RT-qPCR on the culture filtrate. The amount of coronavirus antigen expression in infected cells was measured by indirect immunofluorescence tests, using convalescent serum of patients suffering from SARS-CoV and HCoV-229E infection.[5,6] RT-PCR for SARS-CoV and HCoV-229E was performed directly on culture filtrate according to our previous protocol.[5]

Microarray analysis

Human genome-wide gene expression was examined with the Affymetrix GeneChip system HG-U133A microarray, which is composed of more than 22 000 oligonucleotide probe sets interrogating approximately 18 400 unique transcripts, including 14 500 well-characterised human genes. Quality control, GeneChip hybridisation, data acquisition and analysis were performed at the Genome Research Centre, The University of Hong Kong, according to the standard protocols available from Affymetrix. Data analysis was performed using the Microarray Suite Expression Analysis software (Version 5.1; Affymetrix). For comparison across different arrays, the data for each array were normalised by a global scaling strategy, using a scaling target intensity of 500.

Gene expression analysis by semi-quantitative PCR, quantitative RT-PCR and immunoassays

Genes with significant transcriptional changes known to be associated with biological significance were selected for further analysis by semi-quantitative PCR, RT-qPCR and immunoassays. RT-qPCR was performed according to our previous protocol.[7] The extracted RNA was pre-treated with DNase. Primers that specifically amplified the nine genes related to apoptosis, inflammation and coagulation were designed. The housekeeping gene porphobilinogen deaminase (PBGD) was used to standardise the initial RNA content of a sample. Experiments were performed in duplicate and the results for individual samples were expressed as mean expression level of a specific gene/PBGD relative to the reference cDNA. The relative expression of each infected sample versus the uninfected controls were then calculated and expressed as fold changes. Three sets of immunoassays (human IL8, PAI1 and TFPI2) were performed according to our previous protocols and the manufacturers' instruction.[8,9]

Statistical analysis

The fold changes in the target gene expression and the differences in the concentration of protein expression between SARS-CoV and HCoV-229E at different post-inoculation time points were compared by Student's *t* test. A P value of <0.05 was considered significant. A statistical package (SPSS 10.0) was used for all analyses.

Results

Susceptibility of Huh7 cell line to SARS CoV and HCoV-229E

Using a multiplicity of infection of 100, CPE was visible in Huh7 cells at 24 h and progressed to about 50% cell death at 48 h in both viruses. Both viruses produced a comparable $TCID_{50}$ of around 10^7 per mL in the culture supernatant of Huh7 cells at 48 h. In terms of viral load, one log increase of viral genome copy was noted at 12 h post-infection in both viruses, which was followed by a peak at 24 h. For both viruses, antigen expression could be observed by indirect immunofluorescence in over 50% of the cells at 24 h post-infection.

Effects on gene expression of host cells by microarray

Based on the gene expression analysis, 224 genes were significantly altered within 4 h post-infection. Only 21 genes were disturbed by HCoV-229E per se, whereas 164 genes were altered by SARS-CoV infection only, and the remaining 39 by both coronaviruses. Out of the 164 genes with altered expression in SARS-CoV, 38 were up-regulated and only one was down-regulated at both 2 and 4 h post inoculation. At 2 h post inoculation, 43 were up-regulated and 16 down-regulated. At 4 h post inoculation, 49 were up-regulated and 17 down regulated. In contrast, for HCoV-229E infection, only one gene was up-regulated and no genes were down regulated at both 2 and 4 h post inoculation. At 2 h post inoculation, no genes were up-regulated and only two genes were down-regulated. At 4 h post-inoculation, 14 genes were up-regulated and four were down-regulated. When multiple transcripts of the same gene were eliminated and analysed, genes related to apoptosis (n=23), inflammatory or immune response (n=34) and coagulation (n=5) were identified in addition to the expected genes of stress response, metabolism and other unknown genes. Of the 23 apoptotic genes affected, 13 were pro-apoptotic and 11 were up-regulated in SARS-CoV infection compared to only three in HCoV-229E infection. As for inflammation and immune response, 32 genes were up-regulated in SARS-CoV compared to only three in HCoV-229E. These included NFKB1A, NFKB2, IL8, TGFβ2, chemokines CXCL1, 2, 3, 5, 6 and 10, ICAM1, and TNFα induced proteins. Surprisingly, genes of the pro-coagulation pathway were also affected by SARS-CoV infection with up-regulation of PLSCR1 (phospholipid scramblase 1), EGR1 (early growth response 1 gene), PAI1/SERPINE1 (plasminogen activator inhibitor 1) and THBS1 (thrombospondin 1). In terms of stress response, seven genes were up-regulated in SARS-CoV infection compared to only one in HCoV-229E infection. Overall

there were far more changes in gene expression related to cell cycle, transcription, metabolism, and miscellaneous and unknown functions in SARS-CoV infection. When the Pathway Assist software (Ariadne Genomics Inc.) was used for linking altered genes in cellular pathways for SARS-CoV, there was clear clustering of altered genes related to apoptosis, inflammation and coagulation.

Confirmation of cellular gene and protein expression by semi-quantitative PCR, RT-qPCR and immunoassay

A similar trend to up-regulation of gene expression with SARS-CoV showed a 1.4-10.8 fold increase, compared to HCoV-229E infection for coagulation (TFPI2, PAI1 and THBS1), inflammation (IL8 and NFKB2), transcription (JUNB) and apoptotic (PHLDA1, CARD10 and BAX). Enzyme immunoassay showed SARS-CoV induced higher concentrations of PAI1 and IL8 compared to HCoV-229E at 2, 4 12 and 24 h post-inoculation. Both SARS-CoV and HCoV-229E induced similar TFPI2 expression 4, 12 and 24 h post-inoculation, but at 2 h post-inoculation SARS-CoV induced a lower concentration of this protein.

Discussion

SARS-CoV causes respiratory failure in over 60% of those infected and has a mortality rate of around 15%.[4,10] Apart from pneumonia, occasionally SARS also manifests clinically as pulmonary vasculitis and thrombosis in the lungs among those who died.[11,12] Much has been studied including the virology, genomics, diagnostics, clinical features and progression in relation to viral load, treatment, infection control and immunisation.

No pneumocyte cell line has yet been found to support lytic or non-lytic infection by SARS-CoV. In this study, Huh7 cells were found to be susceptible to SARS-CoV.[13] HCoV-229E produced lytic infection within 48 h post-infection. A high multiplicity of infection of 100 $TCID_{50}$ per cell was used to ensure reproducibility of the gene expression study. Since the expression of a large number of genes was expected to change significantly when virus-induced cytopathology followed a rapidly lytic viral infection, we studied the difference in gene and protein expression profiles at a relatively early stage of infection (ie 2 and 4 h post-infection). This time frame is biologically relevant as proliferation of the Golgi complex and related vesicles and swelling of trans-Golgi sacs were observed in infected cells within the 1st hour of infection. Extracellular virus particles were present in 5% and 30% of the cell populations at 5 and 6 h post-infection, respectively.[14] This also facilitated the analysis as a lower number of altered genes were involved.

Comparative transcriptomic analysis indicated that far more genes (n=136) were up-regulated by SARS-CoV than HCoV-229E. Contrary to the reported findings of increased anti-apoptotic/inflammatory gene expression and decreased pro-apoptotic/inflammatory gene expression in the enterocyte cell lines,[15] far more pro-apoptotic and pro-inflammatory genes were expressed in Huh7 cells infected by SARS-CoV but not HCoV-229E. For instance, expression of BCL2 was induced by SARS-CoV in enterocytes, yet we observed up-regulation of its antagonists, including BAX and BCL2L11, in Huh7 cells. Moreover, much higher expression of other pro-apoptotic proteins, including CASP7, CARD10, PMAIP1, and GADD45B were also induced by SARS-CoV in contrast to HCoV-229E. Furthermore, there was marked perturbation of genes involved in cell cycle regulation, including induction of the CDKN2B gene, which can mediate growth arrest at the G1-phase.

The induction of pro-inflammatory cytokines by SARS-CoV was even more prominent compared to HCoV-229E. The induction of IL8 may be of pathogenic importance as its concentration was positively correlated with disease severity in pulmonary infection with RSV. Thus, the observed significantly higher level of IL8 induced by SARS-CoV in Huh7 cells, compared to HCoV-229E, may recapitulate the host response to these viruses by pneumocytes. The induction of various chemokines of the CXC or CCL families may mediate the chemotaxis of lymphocytes and neutrophils.

These alterations in gene expression are in keeping with the histological changes of SARS hepatitis in which cellular apoptosis, marked accumulation of cells in mitosis with ballooning degeneration of hepatocytes, and moderate lymphocytic infiltration were found in biopsied liver tissues.

The up-regulation of genes involved in pro-coagulation and platelet activation is interesting. TFPI2 inhibits thrombin generation by binding and inactivation of the TF: FVIIa (tissue factor: factor VIIa) complex. Up-regulation of the gene probably represents an inhibitory response to restrain the activation of the coagulation pathway during acute inflammation. In contrast, TFPI2 also inhibits both free and matrix/cell-associated plasmin, thus favouring fibrin deposition and may have a positive role in matrix turnover. Up-regulation of the gene of PAI1 accompanied by a dramatic increase in protein level results in an anti-fibrinolytic response. This may favour fibrin deposition during the acute inflammatory phase of the disease. It is important to note that the mouse hepatitis virus can activate the immune coagulation system by fgl2 gene encoding a prothrombinase. This enzyme can induce macrophage pro-coagulation activity resulting in fibrin deposition on the endothelium of intrahepatic veins and hepatic sinusoids. The result could be confluent hepatocellular necrosis. The low number of liver biopsies performed in these patients may account for the lack of reports on these large-scale changes related to vascular damage. However, systemic vasculitis including oedema, localised fibrinoid necrosis and infiltration by monocytes, lymphocytes, and plasma cells into vessel walls of various tissues has been reported.[12] Thrombosis was found in small veins. Marked up-regulation of a pro-apoptotic gene, PHLDA1, was observed in SARS-

CoV infection of Huh7. Over-expression of this gene in vascular endothelial cells leads to decreased cell adhesion and induces detachment-mediated apoptosis.[16] If similarly induced in vascular endothelial cells infected by SARS-CoV, this gene may contribute to the vascular damage induced SARS-CoV infection.[16]

Conclusions

SARS-CoV produces more severe disturbance of host cell gene expression in a human epithelial cell line of liver origin than the HCoV-229E during the early stage of infection. There are marked alterations in gene expression related to apoptosis, inflammation and pro-coagulation. These findings are consistent with the histological changes of SARS, especially in the liver and blood vessels.

Besides antivirals against SARS-CoV, other modalities of treatment such as anti-apoptotic agents, immunomodulators against inflammation and modifiers of coagulation should be considered in future research on the treatment of SARS. It is important to note that many patients continued to deteriorate 2 to 3 weeks after the onset of SARS, despite a decreasing viral load.

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Prevalence of SARS-CoV antibody in all Hong Kong patient contacts

Key Message

The near absence of transmission (seroprevalence=0.19%) resulting in asymptomatic infection in this representative high-risk group of close contacts indicates that the prevailing SARS-CoV strains in Hong Kong almost always led to clinically apparent disease.

Introduction

Since the SARS outbreak, considerable progress has been made in understanding the biology, pathogenesis, and epidemiological features of both the coronavirus and the disease. Epidemiological studies of hospitalised patients suggest that the overall transmissibility of SARS (as indicated by the basic reproductive number R_0=2.7; 95% confidence interval [CI], 2.2-3.7) is relatively low compared with other pathogens.[1] However, such studies could not take into account possible episodes of mild or moderate illness that did not resort to inpatient care and could not address whether asymptomatic community spread played a role in the 2003 epidemic. If this type of spread occurred, sufficient herd immunity against SARS-CoV to protect against another large-scale outbreak might have developed in the population. The full spectrum of disease associated with SARS-CoV infection should be examined to define more precisely what constitutes a case requiring quarantine and isolation to minimise potential human-to-human spread. Understanding these issues requires the systematic study of the seroprevalence of SARS-CoV antibody in a large sample stratified by age and other baseline characteristics, especially since children were disproportionately less affected by SARS, both in terms of reduced incidence and severity of infection. Serological surveys can be based on a random sample from the total population with appropriate stratification, on serum collected for other reasons (eg blood donors, all hospital admissions), or on surveys of persons who resided in sites of superspreading events or who have had close contact with a confirmed SARS patient.

We report a serological survey for immunoglobulin G (IgG) against SARS-CoV in a representative sample of close contacts of all SARS patients in Hong Kong (>76% had laboratory confirmation of SARS by either paired serology or repeat reverse transcription–polymerase chain reaction [RT-PCR] according to World Health Organization [WHO] criteria).[2]

Aims/objectives

To estimate the seroprevalence and associated predictors of SARS-CoV IgG antibody among all close contacts of the case cohort during the Hong Kong 2003 outbreak.

Methods

During the epidemic (from 15 February to 22 June 2003), close contacts were prospectively identified by the Department of Health through standardised telephone interviews with all 1755 confirmed SARS patients within 1 week of hospital admission. A close contact was defined as a person who had cared for, lived with (in the same household), or came into direct contact with body fluids of the SARS patients within 10 days before hospital admission. A total of 3612 close contacts were recorded; 505 were diagnosed as having SARS. Of the remaining 3107 contacts, 2805 (90%) had a telephone number available, as provided by the primary patient. We successfully contacted 2337 (83%) of the contacts from 23 October to 30 November 2003, and 1776 (57% of those eligible) consented to a telephone interview after the purpose of the study was explained to them by trained public health nurses. The interview consisted of questions that assessed the relationship between the patients and contacts; the timing, intensity

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and frequency of contact; precautionary measures adopted during contact with the patient; known contact with other SARS patients; clinical symptoms of febrile, respiratory, gastrointestinal, or constitutional illness since February 2003; medical and travel history; and sociodemographics. Participants were then invited to provide blood samples for serological testing. Shopping coupons (worth US $25) were given to participants after blood was collected as compensation for time and travel costs.

Samples were screened by the Government Virus Unit of the Department of Health by using viral lysate enzyme-linked immunosorbent assay (ELISA; GBI Biotech, Beijing). Positive results were confirmed with immunofluorescence assay (IFA) and neutralisation tests. For the IFA, microscope slides coated with SARS-CoV–infected FRhK4 cells were incubated with serum samples at serial two-fold dilutions starting from 1:25. A positive test was indicated by cytoplasmic fluorescence under ultraviolet microscopy. Using IFA as the standard, the ELISA detects antibody with IFA titre of >25 (ie sensitivity of 100%) and has a specificity of 95%. Neutralisation tests were performed by standard virological methods with Vero E6 cells and SARS-CoV isolate 6109. A titre of >10 was considered positive. The reported sensitivity of 100% was for convalescent-phase serum samples taken a few weeks after the onset of infection in SARS patients, which should apply to our study. During the early phase of infection, IgM predominates; the ELISA kit we used detects IgG only. Therefore, the sensitivity was 80 to 90% (depending on the number of days after illness onset when the serum samples were taken). However, this sensitivity should not have affected our findings, which were based on tests carried out at least 6 months after the last reported case of SARS in Hong Kong.

Results

Of the 1068 samples analysed, two (0.19%; 95% CI, 0.02-0.67%) contacts had a positive titre (1:25 to 1:50 on IFA compared with at least 1:100 in most recovered SARS cases) for SARS-CoV IgG antibody. None of the two contacts with a positive sample reported a chronic medical condition or being sick with febrile or respiratory illness from February to August 2003. Both seropositive contacts arose from two superspreading events in Hong Kong, ie Prince of Wales Hospital nosocomial outbreak and Amoy Gardens community outbreak.[1,3] The former reported one other close contact, who was interviewed but declined to be tested. The latter was separately identified by three intrafamilial index patients, all of whom lived in the same household and reported only each other as close contacts. The participants who consented to testing were broadly similar to those who declined, except that the former group had relatively fewer children and comprised fewer men. However, those who consented to testing were more likely to report more frequent contact and closer relationships with SARS patients, more febrile or respiratory illness episodes since February 2003, and a travel history to SARS-affected regions, which may have biased our seroprevalence estimate upwards.

Discussion

The extent of seropositivity in close contacts of confirmed patients should provide the upper limit of SARS-CoV antibody seroprevalence in the general population, given the relatively intense exposure of these persons to SARS patients. Our finding of the near absence of transmission resulting in asymptomatic infection in this representative high-risk group of close contacts indicates that the prevailing SARS-CoV strains in Hong Kong almost always led to clinically apparent disease. Whereas some SARS patients (especially health care workers) might have been promptly admitted to hospitals, so that transmission to family members was reduced. Almost all SARS patients (perhaps with very few exceptions in children) had severe disease resorting to inpatient treatment; thus, infection with SARS-CoV almost always caused severe disease requiring hospitalisation.[4]

Although our results suggested that SARS-CoV was a new virus in humans without a close precursor or an antigenically related virus that would have induced at least a small degree of cross-reactivity on serological testing, a recent study on a select group of 938 healthy Hong Kong adults (whose serum had been stored as part of a hepatitis B serosurvey in 2001) indicated that 1.8% of the sample had acquired a SARS-CoV–related virus infection at least 2 years before the 2003 SARS outbreak.[5] The investigators speculated that the virus that affected these healthy, seropositive persons was antigenically closer to the recently isolated animal SARS-CoV–like virus than human SARS-CoV, but interspecies transmission from animals to humans was likely to be inefficient, as the virus might not have adapted in the new host.[3] This hypothesis may explain why only a few persons became infected but were asymptomatic. This hypothesis would be compatible with the presumed asymptomatic infection observed in Guangdong animal traders, especially in those who handled masked palm civets, who had a seropositivity rate of 72.7% (95% CI, 49.8-89.3%) in the absence of prior overt clinical disease.[6]

The limitations of the study included incomplete contact tracing (especially in the earlier parts of the epidemic) and potential recall bias (under-reporting of contacts by some patients who were too sick to answer questions). Another possible shortcoming was the lack of a survey of close contacts whose telephone numbers were not provided, although there was no reason to suspect they had a systematically different serological profile. In fact, these were mostly non-household contacts who would have had less intense exposure to SARS patients. In addition, because peak infectivity, as indicated by viral load, usually occurred during week 2 of illness, when most of the patients would

have been isolated in hospital (the mean symptom onset-to-admission interval decreased from a maximum of 9.3 days in late February to 1.0 day by mid-May). Transmission to close contacts in the later stages of the epidemic was therefore less likely.[7,8] Finally, contacts who refused to participate (n=561) or undergo serological testing (n=708) might have been due to their concerns about having SARS (possibly because of having SARS-like symptoms) and did not want to be identified and stigmatised as having been infected with SARS-CoV. Surveys in other countries with large-scale outbreaks such as Canada, China, Singapore, and Taiwan should be undertaken to confirm our findings.

Conclusions

The near absence of transmission resulting in asymptomatic infection in this representative high-risk group of close contacts indicates that the prevailing SARS-CoV strains in Hong Kong almost always led to clinically apparent disease. It is inferred that infection with SARS-CoV almost always caused severe enough disease requiring hospitalisation.

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Community psycho-behavioural surveillance and related impact on outbreak control in Hong Kong and Singapore during the SARS epidemic

Key Messages

1. The promotion of personal protective health practices must take into account background perceptions of risk and psychological responses in the community-at-large.
2. Population psycho-behavioural factors in Hong Kong and Singapore are shown to be an important potential vector for the transmission of an infectious agent.
3. Comparative psycho-behavioural surveillance and analysis can yield important insights into generic versus population-specific issues that could be used to inform, design and benchmark public health infection control measures.

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Introduction

During a new epidemic such as the SARS outbreak, medical and public health communities focused on identification of the responsible agent as well as pathophysiology, clinical presentation, diagnosis, and treatment of the condition.[1-4] Interest was less in the epidemiology of the disease and the effectiveness of infection control measures in various hospitals; population psycho-behavioural surveillance received almost no research coverage.[5,6] However, formulation and implementation of public health infection control measures deserves equal attention and such recommendations should be based on public perceptions, beliefs and attitudes. Standard data collection and analysis in outbreak control strategies rarely include information about population perceptions about the disease and their relevance to the agent-vector-host epidemiological triangle.

As there may be a possible return of SARS, it is useful to compare the public responses in different cities that were similarly affected. Such comparative analyses enable policy makers to disentangle generic issues from culture-specific concerns and to share practices that successfully controlled the outbreak.

We report a cross-sectional, population-based survey on psycho-behavioural responses to SARS in two centres of the epidemic, Hong Kong and Singapore.

Aims and objectives

To compare public knowledge and perceptions about SARS and the extent to which precautionary measures were adopted in Hong Kong and Singapore.

Methods

Respondents were recruited using random-digit dialling of all land-based telephone lines in Hong Kong and Singapore. A total of 705 Hong Kong (aged ≥18 years) and 1201 Singaporean (aged ≥21 years) residents completed the survey conducted from 15 May to 10 June 2003 in Hong Kong and 5 to 10 May 2003 in Singapore. The respective response rates were 54.7% (705/1288) and 62.3% (1201/1928).

The survey consisted of 60 questions, five of which had multiple parts. It was translated and back-translated from Cantonese to English and vice versa in Hong Kong, and from Cantonese to Mandarin, Malay and English in Singapore. It was pre-tested for face and content validity, length and comprehensibility. The questionnaire was administered in Cantonese in Hong Kong, and in Mandarin, Malay or English in Singapore (at the respondents' choosing).

The respondents were asked: (1) their self-perceived general health status, febrile and respiratory symptoms in the previous 2 weeks, and general anxiety levels using the State-Anxiety Scale of the State Trait Anxiety Inventory (STAI)[7];

(2) their use of health services in the previous 2 weeks; (3) the presence, intensity and setting of direct and indirect contacts with SARS patients; (4) their risk perception in terms of their self-perceived likelihood of contracting SARS and survival if diagnosed with the disease; (5) their beliefs about routes of transmission and confidence in physicians' ability to diagnose the disease; (6) the extent to which various precautionary measures were being adopted and possible changes in lifestyle practices to prevent transmission of the virus; and (7) sociodemographics.

We determined differences in proportions between baseline demographics in this survey and corresponding population statistics in the two cities by calculating the effect size. A value of 0.1 indicates a small effect, 0.3 a medium effect, and 0.5 a large effect. To adjust for possible sampling biases due to sociodemographic differences between respondents and non-respondents and to ensure that the sample was representative of the general populations, we weighted the responses based on the latest figures from the Hong Kong Census and Statistics Department and Singapore Department of Statistics for age, gender and level of educational attainment. All 95% confidence intervals (CIs) were generated using logistic and multinomial regression for dichotomous and multi-categorical variables, respectively.

Using multivariable logistic regression, we sought to identify predictors for greater adoption of a predefined set of precautionary measures (ie at least five of the seven specified strategies) and health services use (defined as presentation to western, Chinese or other complementary and alternative medical practitioners in any setting during the previous 2 weeks). Potential explanatory variables were anxiety level (STAI mean score), level of confidence in physicians' ability to diagnose SARS, self-perceived likelihood of contracting SARS and surviving the illness if infected, presence of physical symptoms, contact history, and sociodemographics. All analyses were conducted using Stata version 8.0.

Results

Comparing the sample demographics with those from the respective population census data, most of the baseline parameters were similar to the benchmark statistics as confirmed by the small effect sizes. To improve generalisability, age, gender and education were used to weight the samples in all subsequent analyses.

Health and emotional status

The anxiety level of Hong Kong respondents was significantly higher than their Singaporean counterparts (mean=2.06 vs 1.77, P<0.001), using the STAI 10-item scale (scores ranging from one [not anxious at all] to four [very anxious]).

Only 0.5% of the Hong Kong and 0.9% of the Singaporean respondents (P=0.36) reported persistent fever of 38ºC for at least 1 day within the previous 2 weeks; about half of whom (0.2% and 0.4%, P=0.47) also had cough or dyspnoea. Respondents with this combination of symptoms was eligible for a SARS diagnosis during an acute outbreak. Hong Kong respondents reported significantly higher prevalences for headaches, difficulty breathing, dizziness, running nose, and sore throat, but none of these (except for difficulty breathing) were cardinal symptoms of SARS. In fact, their presence may have suggested other diagnoses.

When the prevalences of these five symptoms were adjusted for the anxiety level (ie STAI score), they decreased by 7% to 23% in the Hong Kong sample, but remained almost unchanged for the Singaporeans. Given the higher anxiety levels in Hong Kong respondents, psychosomatic presentation may have played a role in the larger proportion of respondents giving a positive response to these symptoms.

Extent of direct and indirect contacts with diagnosed cases and willingness to be quarantined

The majority (92.3% in Hong Kong and 96.7% in Singapore) of respondents reported no contact history, whereas 0.2% of Hong Kong and 0.3% of Singaporean respondents had direct, non-close contact, and 4.1% of the Hong Kong and 1.5% of the Singaporean samples had indirect contact (contact of a direct contact) with a confirmed case. The remaining 3.4% (Hong Kong) and 1.5% (Singapore) of the sample believed they might have been exposed to a possible SARS patient or infected materials (eg fomites).

There appeared to have been a high degree of willingness to comply with quarantine procedures, in the event the respondents were to be exposed to SARS patients. More than 90% of the samples in both cities were willing to be quarantined if there was close (eg household or intimate relationships) contact and at least 70% would be compliant for non-close or social contact.

Knowledge and beliefs about SARS

The majority of respondents in both cities (86.7% in Hong Kong vs 71.4% in Singapore, P<0.001) knew that SARS could be transmitted by person-to-person droplets, although fewer (75.8% in Hong Kong vs 62.1% in Singapore, P<0.001) identified fomites or contact through contaminated objects as a possible transmission mode. These are the two main routes of transmission confirmed by the Centers for Disease Control and Prevention and the World Health Organization. However, 40.9% of the Hong Kong and 50.9% of the Singaporean samples thought that the infection could be transmitted via the airborne route (P<0.001), which does not appear to be the case according to the epidemiological evidence. Overall, Hong Kong respondents were more knowledgeable about the routes of transmission, in terms of the total number of correct responses (P<0.001).

A total of 23% of Hong Kong and 11.9% of Singaporean

respondents believed that they were 'very likely' or 'somewhat likely' to contract SARS during the outbreak (P<0.001). This proportion remained the same even after excluding those who reported any contact (direct or indirect) with a SARS patient. Singaporean respondents were more confident about the ability of physicians to diagnose SARS (29.5% vs 16.1% were 'very confident', P<0.001). However, the corresponding proportions for feeling 'not very confident' or 'not at all confident' were similar in the two cities. Regarding the likelihood of surviving SARS if they contracted the disease, 9.9% of Hong Kong and 11.2% of Singaporean respondents believed their survival was 'not very likely', and 1.9% and 2.2% was 'not at all likely'. Up to the time of the survey, the case fatality rates were 17.1% in Hong Kong and 13.9% in Singapore.

Precautionary measures

The respective proportions of respondents who reported practising each of seven specified precautionary measures (to prevent the transmission and contracting of SARS) directed against the two main modes of transmission (person-to-person droplet spread and fomites) were analysed. There were large differences between Hong Kong and Singapore for six of the seven measures, except for washing hands with soap. Compared with Singaporean respondents, more Hong Kong respondents would cover their mouths when sneezing or coughing (83.6% vs 94.4%) and wash their hands afterwards (72.6% vs 85.6%) as well as after touching possible contaminated objects (48.3% vs 81.2%). About 47.7% of Hong Kong and 27.3% of Singaporean respondents used serving utensils during meals; this is important in Chinese culture, in which dishes are commonly shared with everyone at the table. The difference in proportion of facemask wearing was most striking (79.0% in Hong Kong vs 4.1% in Singapore). At least two thirds of the Hong Kong sample but only 12.6% of the Singaporeans practised at least five of the seven specified preventive strategies.

Predictors for the adoption of precautionary measures and health services use

The level of anxiety (as measured on the STAI scale) demonstrated a positive dose-response relationship with adoption of personal protective measures, especially in Hong Kong (P<0.01). Recent physical health (as inferred from acute respiratory or febrile symptoms) or a contact history with SARS patients was not associated with adoption of precautionary measures. Higher self-perceived likelihood of contracting SARS was a positive predictor in Hong Kong (odds ratio [OR]=1.53; 95% CI, 0.99-2.38), although the results were equivocal for Singapore (OR=1.24; 95% CI, 0.83-1.87). Other variables such as the level of confidence in the ability of physicians to diagnose SARS and the likelihood of surviving SARS did not appear to be predictive. Greater knowledge about the transmission routes of SARS predicted the adoption of more precautionary measures in Hong Kong (OR=2.09; 95% CI, 1.39-3.13). The lack of significant association in Singapore may reflect the much lower adoption of personal protective measures. In terms of sociodemographics, males were much less likely to adopt comprehensive precautionary measures against SARS. There were positive dose-response relationships with increasing age and the level of educational attainment in both cities, where the former relationship was stronger in Singapore and the latter in Hong Kong. To assess whether anxiety level was an intermediary between risk perception and uptake of precautionary measures, we re-analysed the model while omitting the STAI score as an independent variable. This revealed that the OR estimates for the two self-perceived likelihood factors did not change appreciably, thus confirming that anxiety was not a significant intermediary causal factor.

The presence of symptoms was the only robust predictor for higher health services use. Respondents' health-seeking behaviour did not appear to have been influenced by extraneous factors such as risk perception, anxiety level or contact history. However, younger, male respondents were less likely to seek health care services.

Discussion

This population-based, cross-sectional survey revealed substantial differences in the knowledge, beliefs, emotional status, and extent of adopting personal protective measures between Hong Kong and Singapore at the end of the SARS epidemic. Areas of commonalities between two cities included levels of civic compliance with public health control and quarantine directives, as well as predictors of greater adoption of precautionary steps and health services use. Public health action to curb the transmission of SARS coronavirus was mainly effected through enhanced personal hygiene and health protective measures. This was dependent on the public knowledge, psychological responses (viz anxiety level) and the perceptions of the community-at-large. There were sociodemographic subgroups that were less likely to take personal protective steps or to seek care. The strength of this study was that respondents were interviewed during an actual outbreak, compared with other studies of infectious disease epidemics or bioterrorism attacks in which hypothetical questions were usually posed.

As the survey was conducted during the epidemic (close to the end), knowledge indices were expected to be at their highest given the cumulative effects of sustained promotion of health practices through mass media. Nonetheless, there were still significant knowledge gaps in terms of the routes of SARS coronavirus transmission (more so in Singapore than Hong Kong). In addition, respondents' risk perception as indicated by their perceived likelihoods of contracting and surviving SARS were exaggerated and overly pessimistic when benchmarked against the overall probabilities based on the numbers of patients infected and died. This could be explained by a combination of knowledge deficits and excessive anxiety generated by the outbreak, although the

present analyses preclude drawing of definite conclusions.

The stage of the epidemic at which we conducted the survey could have affected our observations regarding public behavioural responses. Singapore's lower adoption prevalence of precautionary measures might arguably have been due to the low daily new case counts at the time of the survey, although it would have been difficult for the population to foretell this given that in Singapore similarly low new daily counts were observed towards the end of March and early April, only to peak again 2 weeks later. Toronto also experienced a similar bimodal distribution of cases. The Hong Kong survey was also carried out during the end of the outbreak, but a much larger proportion of respondents reported continued vigilance for personal protective precautions and more comprehensively. Assuming this cross-sectional pattern was representative of the entire epidemic in both cities and that there was no ecologic fallacy, the very different extent of the respective outbreaks in Hong Kong and Singapore must be due to other factors. For instance, the impact of the two superspreading events at the Prince of Wales Hospital (n=239) and Amoy Gardens (n=329) in Hong Kong (where the former 'seeded' the latter) might have dominated over the much smaller effects of community transmission (where one infected individual typically spread the disease to three others in the absence of any preventive measures), which was dependent on public collective adoption of personal preventive measures. This hypothesis, if substantiated, underlines the often stochastic or random nature of such epidemics.

Our findings have important implications for public health and infection control. Public health messages in providing appropriate advice and education during this epidemic were highlighted. There were significant gaps in the public knowledge about SARS such as the route of transmission and risk perception, which were associated with inadequate adoption of precautionary measures. Therefore, health education and promotion efforts should be stepped up to prepare for a possible return of SARS.

Anxiety can be either a facilitator or barrier for promoting adoption of precautionary measures. This study confirmed that the population attitudes and perception of events were important indices. They should be closely monitored during an outbreak like SARS, as they can be highly predictive of key behaviours.

Younger, less-educated males (ie traditional risk-takers) were least likely to adopt appropriate preventive measures. Targeting health promotion messages through intermediaries such as female significant others (eg mothers, wives or girlfriends) who are more health conscious and risk averse may raise the level of protective precautions undertaken by this vulnerable subgroup.

Only those with symptoms were more likely to seek medical attention. Other factors, such as risk perception and anxiety level, did not significantly influence health care use, suggesting that there was little detectable panic or irrational use of health services in both cities. This could have been due to avoidance of health care facilities by the public to minimise exposure to high-risk areas (hospitals) and health care personnel. Nonetheless, panic and irrational use of health services during large outbreaks could in theory overwhelm any health care system.

The limitation of this survey was that it was administered at a single time point such that the stability of the responses is unknown, although in Hong Kong repeated cross-sectional and time series data as well as prospective panel data at various points of the epidemic were collected. The analysis of this longitudinal data set can track possible psycho-behavioural changes as epidemics evolve and evaluate the macro impact of policy decisions. In addition, the use of structural equation modelling linking different psycho-behavioural variables to better delineate the causal chain of events deserves further examination. Further exploration of public beliefs and their interplay with traditional health beliefs and practices would be a useful adjunct to understand population psycho-behavioural responses. Such qualitative research should be a high priority to prepare for future large-scale epidemics.

Conclusions

Promotion of personal protective health practices must take into account background perceptions of risk and psychological responses in the community-at-large. Population psycho-behavioural factors in Hong Kong and Singapore were a potential vector for the transmission of an infectious agent. Comparative psycho-behavioural surveillance and analysis can yield important insights into generic versus population-specific issues. Such issues could be used to inform, design and benchmark public health infection control policy measures.

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Influenza-associated hospitalisation

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Key Message

The disease burden associated with influenza includes not only acute respiratory diseases but also cerebrovascular disease, ischaemic heart disease and diabetes mellitus.

Introduction

Influenza may result in hospitalisation, especially in young children and the elderly or in those with chronic diseases. Hospitalisation for influenza is under-reported owing to the non-specific symptoms of influenza and lack of laboratory tests. Excess hospitalisation and mortality associated with influenza have been used to measure the health impacts of influenza.[1-3] Excess hospitalisation was calculated as the difference in hospitalisation numbers between the epidemic and non-epidemic periods within a year, or between the epidemic years with apparent influenza peaks and the baseline years without any apparent increase of influenza activity. It is difficult to apply the same approach to tropical and subtropical regions such as Hong Kong, where there are no well-defined epidemic periods or even clear seasonal patterns of influenza activity. We used Poisson regression to estimate the disease burden related to influenza in the circumstance without a clear influenza seasonality.[4]

Aims and objectives

To evaluate influenza-associated hospitalisation for different age-groups in Hong Kong.

Methods

The weekly numbers of hospital discharge diagnoses from 14 acute hospitals in Hong Kong during the period 1996-2000 were obtained from the Hospital Authority. The disease categories included acute respiratory disease (ARD) [International Classification of Diseases version 9 (ICD9) codes 460-466, 480-487] and its sub-category pneumonia and influenza (P&I) [ICD9 480-487], cerebrovascular disease (CVD) [ICD9 430-438], ischaemic heart disease (IHD) [ICD9 410-414] and diabetes mellitus [ICD9 250]. The weekly proportion of specimens positive for influenza A and B (influenza A+B), and for respiratory syncytial virus (RSV) were obtained from the microbiology laboratory of Queen Mary Hospital and were adopted to represent influenza and RSV activity.

We used Poisson regression to model the weekly counts of hospitalisation for each disease category.[5] We built a core model to control for confounding factors including temperature, relative humidity, long-term trends and seasonality. This core model was accepted once the partial autocorrelation plots of its residuals did not have any discernible patterns. The variables influenza A+B and RSV were entered into the core model to assess the impacts of influenza activity with adjustment for RSV. The lag effects of influenza were assessed by entering the variable influenza A+B measured at 0-3 weeks before the admission week and selecting the one with the most significant effect (ie the smallest P value for the coefficient).

The percentage of excess hospitalisation attributable to influenza was calculated as the ratio of the difference between the total observed and expected hospitalisation under the assumption that influenza A+B was zero, to the total number of observed hospitalisation. This percentage of excess hospitalisation was then multiplied by the total hospitalisation per year to get the excess number, which was further divided by the population to obtain a rate of excess hospitalisation per 100 000 inhabitants.

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Table. Influenza-associated excess hospitalisation in Hong Kong from 1996 to 2000, after adjusting for co-variates including respiratory syncytial virus

Age-group (years)	Lag (weeks)	Excess hospitalisation per year		P value
		% (95% CI)	No. (95% CI) per 100 000 inhabitants	
Acute respiratory disease				
0-14	0	9.3 (7.7, 10.8)	163.3 (135.2, 189.7)	<0.001
15-39	0	7.2 (3.3, 10.8)	6.0 (2.7, 8.9)	0.001
40-64	1	11.0 (7.9, 13.9)	14.9 (10.7, 18.8)	<0.001
65-74	1	11.5 (8.4, 14.3)	83.8 (61.2, 104.2)	<0.001
75+	0	8.7 (6.5, 10.8)	266.0 (198.7, 330.2)	<0.001
All	0	10.9 (9.5, 12.1)	60.6 (52.8, 67.2)	<0.001
Pneumonia and influenza				
0-14	1	14.7 (11.8, 17.4)	70.4 (56.5, 83.3)	<0.001
15-39	1	10.5 (6.1, 14.4)	2.9 (1.7, 4.0)	<0.001
40-64	1	8.7 (5.0, 12.1)	6.8 (3.9, 9.4)	<0.001
65-74	1	11.0 (8.1, 13.8)	58.7 (43.3, 73.7)	<0.001
75+	0	7.1 (4.8, 9.3)	176.3 (119.2, 231.0)	<0.001
All	1	11.6 (10.2, 12.9)	29.3 (25.8, 32.6)	<0.001

Results

In all age-groups, influenza was significantly associated with 10.9% of total hospitalisation for ARD, 11.6% for P&I, 1.5% for CVD, 1.8% for IHD and 3.5% for diabetes (P<0.01) [Table]. In addition, ARD and P&I were significantly associated with influenza (P<0.001). Influenza accounted for 9.3% and 11.5% of hospitalisation for ARD in the 0-14 and 65-74 years age-groups, respectively. For both disease categories, the influenza-associated excess hospitalisation rates per 100 000 inhabitants were estimated to be lowest (6.0 for ARD and 2.9 for P&I) in the 15-39 years age-group, and highest (266.0 for ARD and 176.3 for P&I) in the 75+ years age-group (Table).

Influenza was significantly associated with hospitalisation for CVD (P=0.001) and IHD (P<0.01) only in the 75+ years age-group, with rates of 55.4 and 56.4 per 100 000 inhabitants. Influenza was also significantly associated with 2.0% of all hospitalisation for IHD in the 40-64 years age-group (P<0.05). In addition, influenza was significantly associated with hospitalisation for diabetes (P<0.01), with rates being 6.6, 23.9 and 53.3 per 100 000 inhabitants in the 40-64, 65-74 and 75+ years age-groups, respectively. For all these chronic diseases CVD, IHD and diabetes, the rates of influenza-associated excess hospitalisation were highest in the 75+ years age-group (Fig).

Discussion

In this study, influenza was significantly associated with cardiorespiratory hospitalisation in all age-groups in Hong Kong. According to our estimates, influenza accounted for 29.3 excess P&I hospitalisations per 100 000 inhabitants in all age-groups and 11.6% of total hospitalisations. These estimates were comparable with those reported by a study in the US using a similar Poisson regression approach. Annually there was a 36.8 influenza-associated excess P&I hospitalisations per 100 000 inhabitants for all ages, accounting for 8.6% of all hospitalisations for this diagnosis during the period 1979 to 2001.[6] Those aged 75+ years had the highest hospitalisation rates for all disease categories investigated. The influenza-associated hospitalisation for ARD was lowest in the 15-39 years age-group, a pattern echoed in the US study.[6]

Influenza was associated with excess hospitalisation for chronic diseases such as CVD and IHD, especially in those older than 65 years. Numerous studies have suggested that influenza infection may cause damage not only to the respiratory system, but also to the circulatory organs. Influenza might trigger alterations in circulating clotting factors, platelet aggregation and lysis, concentrations of inflammatory-response proteins and cytokine concentrations. These may enhance thrombotic tendencies, impair vasodilation, or even lead to endothelial injury. In addition, acute respiratory tract infection is associated with an increased risk of myocardial infarction and stroke.[7]

The association between influenza and hospitalisation for diabetes was significant. Our study was the first

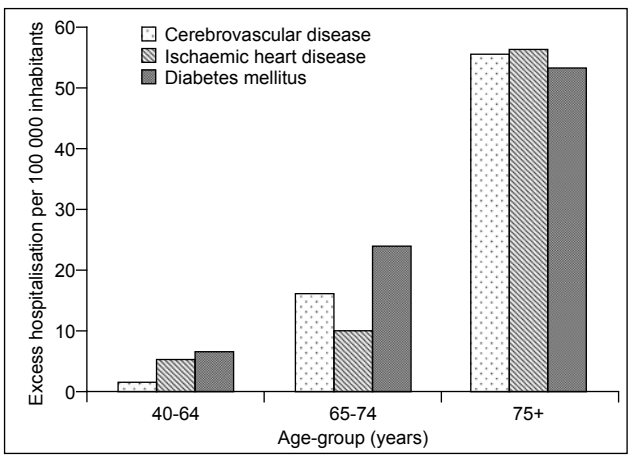

Fig. Excess hospitalisation per 100 000 inhabitants associated with influenza at different age-groups

showing that influenza was associated with hospitalisation for diabetes mellitus in persons older than 40 years. Although increased morbidity and mortality of diabetes during influenza epidemics has long been recognised, we were able to assess the excess hospitalisation rates due to year-round influenza virus activity rather than in epidemic periods alone. The increased hospitalisation of diabetic patients may be explained by their impaired immune responses, which probably leads to secondary bacterial infections and further increases morbidity and mortality. Moreover, influenza and its complications may increase the chance of diabetic patients suffering a range of diabetes-related complications that may or may not be directly related to influenza.

Young children and the elderly are high-risk groups in tropical and subtropical settings; they would most benefit from influenza vaccination. Influenza also significantly increased the risk of hospitalisation in patients with underlying chronic conditions such as CVD, IHD and diabetes. These results provide evidence to support the promotion of influenza vaccination in patients with these chronic diseases.

Poisson regression modelling is a valid approach to assess disease impacts of influenza in tropical and subtropical regions with uncertain seasonal patterns of virus activity. The estimates based on Poisson regression modelling have been proved to be robust to unobserved confounding factors such as smoking status and chronic co-morbidity.[8] However, this methodology has the limitation of over-controlling underlying seasonal variations of disease morbidity in the core model. It is possible that some of this seasonal variation may also be attributable to the seasonal activity of influenza, but this portion of the variation cannot be separated from others in this type of analysis. Therefore, our estimates are very likely to underestimate the true impact of influenza on hospitalisation. An approach that can also capture the seasonal variation of disease morbidity associated with influenza virus circulation is needed.

Conclusions

Our study demonstrated an association of influenza with hospitalisation, not only for ARD (including P&I), but also for chronic diseases such as CVD, IHD, and diabetes mellitus. The excess hospitalisation rates associated with influenza in Hong Kong were estimated to be close to the rates in the US.

Influenza vaccination in the tropics should be promoted to achieve a direct benefit of reducing influenza-associated hospitalisation. The Poisson regression approach is applicable in estimating disease burden associated with influenza in Hong Kong, and can be adapted to other subtropical/tropical regions.

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Reducing the impact of the next influenza pandemic using household-based public health interventions

Key Message

Household-based public health interventions can effectively mitigate the impact of influenza pandemic, and the resources and compliance requirement are realistic and feasible.

Introduction

Wherever a pandemic influenza strain evolves, there is a period of time during which the disease has not yet reached some populations. This allows these populations to implement interventions to reduce local transmissibility (measured by the basic reproductive number R_0) prior to the introduction of the strain. This may reduce the infection attack rate (IAR) once the pandemic arrives. We estimated the reduction in IAR after different household-based interventions using a mathematical model of influenza transmission within and between households. Household-based interventions, such as voluntary quarantine and antiviral prophylaxis, may reduce the IAR substantially, without consuming resources at the same rate as non-targeted population-level interventions. To estimate the impact of household-based interventions on IAR, we used an individual-based stochastic model of influenza transmission with explicit household, peer-group, and community settings.

Aims and objectives

To estimate the effectiveness of preventive measures that communities might implement to reduce the impact of pandemic influenza.

Methods

In this simulation, the distribution of household sizes and the average numbers of children in households of different sizes were made to be consistent with Hong Kong.[2] All interventions were active prior to the arrival of the infected individuals, and the population had a constant introduction of 1.5 infected individuals per day per 100 000 inhabitants for 365 days. Susceptible individuals reported with influenza-like-illness, caused by something other than the pandemic influenza strain, at a constant rate of 74 per day per 100 000 inhabitants (according to Hong Kong Centre for Health Protection, www.chp.gov.hk, Data and Statistics, Sentinel Surveillance).

Household-based interventions were simulated as an integrated process of voluntary household quarantine, voluntary individual isolation, and contact tracing. Quarantine referred to segregation of household contacts of a suspected patient from other members of the community within their own homes. Isolation referred to relocation of symptomatic individuals from their household to a separate facility. If an individual complied with household quarantine, his infectivity to other household members increased by a factor of ε_0 (ε_0=2 at baseline). Also, the level of transmission in isolation may be higher than elsewhere. The degree of transmission in isolation was assumed to be a factor of ε_1 greater (ε_1=1 at baseline). Individuals with symptoms severe enough to warrant hospitalisation were assumed to be isolated and to receive antiviral therapy. Compliance was modelled at the individual level (ie each member of the household made independent decision). We defined p_c to be the probability of compliance. These interventions were implemented using the following algorithm:

(1) An individual from households not in voluntary quarantine had the opportunity to enter the programme via one of the following three routes: developing symptoms, being contacted through contact tracing, being hospitalised.

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We assumed that the subject actually reported with a probability p_c for symptoms and contact tracing, and with probability 1 for hospitalisation, and complied with the programme until released. After release, the subject was not bound by previous decisions to join or not join, ie being able to choose once again.

(2) Each of the other members of the household complied with intervention instructions with a probability p_c.

(3) After a delay of 1 day, all compliant non-symptomatic household members took one dose of prophylactic antivirals per day, when antiviral policies were in effect. Symptomatic household members took two doses of anitvirals per day.

(4) If contact tracing was in effect, each compliant adult member of the household would name, on average, five subjects (in their peer-group).

(5) If isolation was in effect, new symptomatic individuals who were compliant would enter voluntary isolation with a probability p_c after a delay of 1 day. If the isolated individuals no longer showed symptoms after 3 days, they would be released from isolation and rejoin their household, which might be quarantined. Otherwise, they would be isolated for a further 3 days. This cycle would be repeated until the subjects no longer showed symptoms or died.

(6) Isolated individuals were given two doses of antivirals per day, without a delay, in all simulations, regardless of the policy for the use of antivirals in households.

(7) If contact tracing was in effect, contacts (whether known or not already in the programme) of all new symptomatic or hospitalised household members would be traced with a mean delay of 1 day.

(8) In the absence of new symptoms in compliant or hospitalised household members for 7 days, the quarantined household would be released from the programme at that point. Otherwise, they would return to step 5.

Results

With compliance rates of 50%, all intervention policies substantially reduced IAR. The baseline IAR of 74% was reduced to 49% when voluntary household quarantine was in effect. However, the peak proportion of households that were quarantined, even with compliance rates of only 50%, was 9.6%. The addition of voluntary individual isolation further reduced the IAR to 43% and the peak proportion of households that were quarantined decreased to 7.1%. Voluntary individual isolation provided an incentive for households to participate: presumed infectious individuals may have been prioritised for health care services and would have protected household members. However, this approach required isolation facilities for up to 0.9% of the population at the peak of the epidemic.

We also considered the use of antivirals with voluntary household quarantine. This policy had a similar efficacy to voluntary individual isolation (IAR, 44%) at a cost of 3.9 doses of antiviral per member but with a much smaller peak level of isolation of 0.5%. The use of antivirals in addition to quarantine and isolation further reduced the IAR to 40% and the peak proportion of households that were quarantined reduced to 6.2%. The addition of contact tracing reduced the IAR to 34% but increased the proportion of the population in quarantine considerably. The additional requirements of contact tracing are unlikely to be justified unless the reproductive number is reduced to near one by other interventions. The prevalence of quarantine and isolation specifies the resources required by these programmes over time, eg the total prevalence of quarantine and isolation on a given day indicates the number of antiviral doses that needs to be distributed, when the use of antivirals in addition to quarantine and isolation is in effect.

As the influenza strain that may cause the next pandemic has not yet been observed, it is not possible to estimate its level of transmissibility (other than by using historical data from other strains) or the balance of transmission in different settings.[1] We used extensive Latin hypercube sampling to conduct sensitivity analyses. This suggests that variations in the efficacy of policies in reducing the IAR is dominated by the basic reproductive number R_0.[3] All interventions are considerably more cost-effective for lower values of R_0.

The efficacy of quarantine plus antivirals was not substantially less than that of quarantine, isolation plus antivirals for most parameter combinations. The potential for increased transmission in isolation did not seem to substantially decrease the efficacy of the voluntary individual isolation. Even with isolation transmissibility levels 10 times greater than those outside isolation, the voluntary individual isolation was still effective (IAR, 45%), compared to voluntary household quarantine alone (IAR, 49%), because the overall proportion of susceptible individuals entering isolation was low. Although this proportion may have been high during the initial stages, it would likely be small when averaged over the entire course of the epidemic.

All estimated reductions in IAR were sensitive to the population compliance rate, p_c and to the proportion of transmission, θ, which was either asymptomatic or pre-symptomatic. Values of p_c=50% and θ=30% were assumed for baseline intervention scenarios. Our estimated changes in IAR were also sensitive to the average delay in the provision of antivirals and in voluntary isolation, although less so than to p_c and θ. In deciding whether to implement any or all of the policies described, local public health officials may wish to consider available epidemiological data (to assess R_0 and θ) and also estimate the levels of compliance that could be achieved for the different options in their populations. As compliance may be higher for policies that provide immediate benefits to the individual, compliance will be low for voluntary household quarantine alone, higher for voluntary individual isolation alone, and the use of antivirals with voluntary household quarantine, and highest

for the use of antivirals with voluntary household quarantine and individual isolation. It is likely that the provision of antiviral prophylaxis and treatment increases compliance substantially. Our baseline assumption of 50% is intended to be conservative. It seems that household-based interventions work when levels of compliance are high. Even moderate levels of compliance render household-based public health interventions effective. Also, the marginal benefits from the use of antivirals and isolation may not be justified if the average times for provision of these services exceed 3 to 4 days, given that the quarantine period is set at 7 days.

Levels of compliance with quarantine and isolation would likely improve in the early and late stages of the epidemic, when a viable diagnostic method is available. We considered the impact of virological testing as a diagnostic support for these policies. However, current low throughput (limited by both laboratory infrastructure and supplies of reagents) and low test sensitivity (due to difficulties in obtaining adequate specimens outside of specialised care settings) meant that it was not a worthwhile addition. If an inexpensive, easy-to-perform, rapid and accurate test was available, it would have a significant impact on transmission and on peak levels of quarantine, when used as part of a wider household-based programme.

Discussion

For lower transmissibility strains of pandemic influenza, the combination of voluntary household quarantine, individual isolation, and the prophylactic use of antivirals was highly effective and feasible across a range of transmission scenarios, even with only moderate levels of compliance. We have quantified the resources consumed by this and similar policies in terms of numbers of people quarantined, numbers of people isolated, and doses of antivirals required.

We assume that the natural history of the next pandemic strain will be similar to that of the 1918 strain, a reduction in IAR from 74% to 40% would avert 16 000 deaths during the period of the initial pandemic wave in a city about the size of Hong Kong (6.8 million people). Our results suggest that such a reduction can be achieved using the combination of voluntary quarantine, individual isolation, and antiviral therapy. Isolation on such a large scale may be somewhat controversial, given the infrastructure requirements of such a policy. Therefore, when large stockpiles of antivirals are available, the marginal benefit of the additional use of isolation may not be justified. However, when stockpiling of antivirals is not feasible, individual isolation is the best possible addition to household quarantine.

Our results build on previous modelling studies of pandemic influenza which focus on the possibility of containment using geographically targeted antiviral therapy.[1,4] Effective strategies have been identified for mitigation rather than containment.[5,6] The key outcome of mitigation is the reduction in IAR, rather than the likelihood of complete control. Given that many epidemiological parameters associated with the next influenza pandemic are unknown, comparison of results from different modelling studies is not straightforward. Our results are consistent with the reduction in IAR from 34% to 20%.[5] However, they are not consistent with other studies, in which a 10 fold reduction in the numbers of ill people is reported for the use of targeted anti-viral prophylaxis.[6] This large discrepancy is likely due to the optimistic nature of their policy: they assume that households, household clusters, schools and workplaces can be targeted very efficiently for prophylactic antiviral therapy. We suggest that a highly efficient contact tracing process be required to achieve high levels of coverage between socially connected households, which is particularly true in modern urban populations. Such a process requires large numbers of households to be recruited during short periods of time, which is not feasible.

Reducing the first-wave IAR should be the primary goal of influenza preparedness planning. When complete transmission control is not achieved, this necessarily implies a longer epidemic. If the mortality rate of the pandemic strain is considered to be low, it is likely that some governments will place priority on reducing the duration of the outbreak than on reducing the number of infections. For a longer period of societal disruption, policy should be designed to reduce mortality and peak stresses on the society as a whole. For example, for the baseline case, a combination of voluntary household quarantine, individual isolation, and use of antivirals could reduce the peak incidence of infection from 3.7 to 0.8%. Although such analyses are beyond the scope of this work, the likelihood of maintaining uninterrupted key societal services (such as law enforcement, food distribution and utility provision) may improve substantially across this range. Therefore, the potential massive adverse economic implications of a temporary breakdown may justify extending the expected period of disruption.

Conclusions

Household quarantine was not successfully implemented on any significant scale during the 1918 city-level epidemics upon which estimates of transmissibility are based.[1,7] Therefore, the likely impact of the interventions we described is real and not already incorporated into estimates of transmissibility. Modern transport and communication infrastructures are much more advanced than those available in 1918, so it is reasonable to expect that such interventions can now succeed. Many countries have put in place formal pandemic preparedness plans following a World Health Organization framework. These national plans mention the interventions included here, but they do not specify the implementation of intervention processes in even the broadest terms, nor do they attempt to predict the levels of resources required. Our findings and future studies, which match detailed descriptions of

interventions with realistic transmission models, can help to inform pandemic preparedness plans by quantifying both the benefits of, and resources required by, household-based interventions against pandemic influenza.

Our measures to increase social distance consume substantial resources and therefore detailed planning is required. To allow quarantined individuals to remain at home, provision of food, water and medicines must be made for. This may be achieved through a central system or a neighbourhood assistance scheme. For isolation, careful planning and investment is required so that large facilities can be made operational in time to reduce transmission in the early stages of the epidemic. For antivirals to be provided efficiently, a dedicated distribution system is required.

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Knowledge of risk and self-protection practices and the degree of influenza hazard from live poultry sales

Key Messages

1. Perceptions of risk from buying live chickens were moderate, but sickness anxieties did not predict buying or touching habits.
2. Buying was strongly predicted by the erroneous belief that cooking is the best means of protection from avian influenza. Health education groups seeking to increase preventive practices to control possible avian influenza outbreaks need to learn from this.

Introduction

Pandemic human influenza strains emerging from co-infection of a human influenza carrier by avian influenza H5N1 virus is a small risk, but the public health impact could be catastrophic. Low probability, highly prevalent events have considerable public health importance.

Domestic waterfowl, chickens, and pigs act as aberrant hosts for both avian influenza (from migratory waterfowl and shorebirds) and human influenza viruses. Genetic reassortment of influenza viruses is likely to be more rapid in aberrant hosts.[1] Domestic animal and human avian influenza infection may therefore increase the chance of a potentially pandemic strain emerging.

Most human-animal contact is domestic or commercial. Most human avian influenza infections occur among persons working or living with domesticated birds.[2] Wet markets provide opportunities for people and live animal mixing, making them potential sources of viral amplification and infection. Severe acute respiratory syndrome coronavirus probably emerged in wet markets. Direct hand-to-face contact is the most likely path for infection. Highly dense urban populations increase opportunities for infection and transmission in any outbreak.

Minimising unnecessary mixing between people and domestic poultry by replacing live animal sales in wet markets with hygienic central slaughtering and chilling is therefore valuable.

Aims and objectives

To determine population knowledge of risk self-protection practices and to estimate the degree of influenza hazard from live poultry sales at the height of the 2004 Asia avian influenza epidemic.

Methods

A telephone survey of the general population was performed from 10 am to 10 pm from mid-February to mid-March 2004. Households were selected by using random digit dialling. Within households, respondents were selected by using random number tables based on varying household sizes. Inclusion criteria were Cantonese speakers, age of 16 to 95 years, and residing in Hong Kong for >12 months.

Instrumentation

Of the six-section questionnaire, three sections are addressed here. Section 1 consisted of Likert scale items assessing self-rated health (excellent to very poor) and influenza-like symptoms (fever, chills, cough, headache, myalgia, breathing difficulties, coryza, sore throat, diarrhoea and low back pain ['yes', 'no', 'don't know']).[3] Section 2 consisted of 13 questions on household practices when buying live birds, and three of them assessed risk perceptions: worries about catching avian influenza from buying live chickens, likelihood of self/family members getting sick from buying live chickens (all using five- or seven-point

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categorical ordinal response formats) and a decile anchored 0% to 100% probability assessment of getting sick from buying live chickens.[4] To help identify attitudinal and knowledge predictors of risk perceptions and behaviour change, respondents expressed agreement or disagreement using five-point Likert scales (strongly agree to strongly disagree) with 32 statements addressing attitudes, avian influenza protection practices, and perceptions of live chicken sales. Section 3 consisted of nine items concerning demographic information.

Data analysis

Categorical data were analysed with Chi squared tests and continuous data with *t* tests. Average annual live chicken purchase rates were calculated by using a conservatively estimated number of live chicken purchases per response category. To households reporting one live chicken purchase per year, one live chicken purchase was attributed; to households reporting 'a few times a year', four were attributed; to households reporting 'monthly', 12 were attributed; to households reporting 'a few times per month', 24 were attributed; to households reporting 'weekly', 52 were attributed; and to those reporting 'a few times a week', 100 were attributed. Perceived risk moderates behaviour. To identify predictors of greater risk perception and behaviour, purchase (yes/no) [model 1] and touching during purchase (yes/no) [model 2] of live chickens, and perceived likelihood of getting sick from buying live chickens (dependent variable 50th percentile dichotomised 0% to 100% probability assessment responses to the question, "How likely is it that you will get sick from buying live chickens?") [model 3] were regressed in forward-stepped multivariate logistic equations on five attitudinal factors, adjusted for demographics. Attitudinal factors were derived by reducing the 32 attitudinal statements with varimax-rotated principal components factor analysis by using scree-plot and Eigen vector-driven factor extraction. Dichotomisation and logistic regression were required for binary dependent variables in models 1 and 2, and to overcome multimodal distribution difficulties on the response scale used in model 3. All proportions were rounded to the nearest whole number. Analyses were performed using SPSS 11.0. (SPSS, Cary [NC], US).

Results

Seven interviewers called 6603 telephone numbers in 4 weeks. Of these, 2596 were invalid (fax or answering machines), and persons reached by 1765 numbers were ineligible (non-Cantonese speakers, residing in Hong Kong for <12 months). Of 2240 eligible respondents, 1256 declined to participate or complete the survey (556 were 'too busy', 688 refused for other reasons), leaving 986 eligible respondents who completed the survey, giving a response rate of 44% (986/2240).

The sample comprised 589 women and 397 men closely matching the most recent population census data. Men had a wider age distribution than did women (P=0.006), were more likely to be single (P<0.001), born in Hong Kong (P<0.001), and better educated (P=0.015).

Purchase of live chickens

Of female respondents, 20% (116/589; 95% confidence interval [CI], 17-23%) reported that their household never bought live chickens, compared to 24% (96/396; 95% CI, 20-28%) of male respondents. In households (78%) that reported buying live chickens, 76% (95% CI, 72-78%) of female and 31% (95% CI, 26-36%) of male respondents did so personally; other family members or domestic helpers did the rest of the purchasing. Of male respondents, 18% (95% CI, 14-22%) reported that all family members bought live chickens, 14% (95% CI, 10-18%) claimed to be the sole purchasers, whereas 69% (95% CI, 64-74%) reported that other household members did the purchase. The corresponding rates among females were 11% (95% CI, 8-14%), 65% (95% CI, 61-69%), and 24% (95% CI, 20-28%).

Because 65% of women but only 14% of men personally bought live chickens, we adjusted for sex differences in purchasing rates by applying the female rate to the remaining proportion of purchases in male-respondent households (86%), and all but 14% in female respondent households, the remainder being attributed at the male rate.

Contact with live chickens during purchase

Of the 78% of respondents who reported their household bought live chickens, 13% (95% CI, 10-16%) of female and 19% (95% CI, 14-23%) of male purchasers touched the chickens when buying. Overall, 14% (95% CI, 9-13%) of purchases involved physical contact with a live chicken. Extrapolating these exposures (14% of 78%=11%) by the average number of chickens purchased annually (18.7), multiplied by the number of Hong Kong households (2 051 890), gives 4 220 738 person-chicken exposures annually. Of those reporting that they touched live chickens when buying, only about 30% said they 'always' or 'usually' washed hands afterwards. Anxiety scores did not differ between those who bought live chickens and those who did not.

Risk perception

Among all respondents, four separate items tapped perception of risk from buying live chickens. The first assessed perceived objective risk. Overall, 36% (95% CI, 33-39%) of respondents agreed with the statement 'buying live chickens is risky to health'. The next two items considered perceived consequences of risk (odds of getting sick). Statement-based probability estimates for 'getting sick from buying live chickens' indicated that 34% (95% CI, 31-37%) of respondents considered that they would 'never' or were 'very unlikely' to get sick from buying live chickens, whereas 27% (95% CI, 24-30%) thought it was 'unlikely', 24% (95% CI, 21-27%) 'chances are even' and 15% (95% CI, 13-17%) 'likely' or 'very likely'. The

third item (0-100% probability estimates of sickness risk) produced lower risk estimates than the second item, with 53% (95% CI, 50-56%) perceiving the likelihood of getting sick at below 26%, 38% (95% CI, 35-41%) in the range 26-50%, and 9% (95% CI, 7-11%), exceeding a 51% likelihood. Item 4 assessed the risk expressed by others. Overall, 46% (95% CI, 43-49%) of respondents reported that their friends had expressed worries about catching avian influenza. Risk perceptions did not differ by age, sex, education, income, or occupation.

Factor analysis

The 32 attitude statements produced a five-factor best-fit solution, which accounted for 38.5% of the score variance. These five factors were labelled according to their item content. Factor 1, 'animal husbandry risk' (10% of variance), included items attributing avian influenza to market practices, live animal sales, and poor home and market hygiene. Factor 2, 'traditional market practices' (9% of variance), items supported traditional markets, their low health risks, live chicken sales, and trivialised health 'scares'. Factor 3, 'protective practice' (8% of variance), items reflected unwillingness to continue live chicken purchases despite risks, unwillingness to take risks for enjoyment, risks from zoonotic infections, and responsibility for own health. Factor 4, 'avian influenza anxieties' (6% of variance), items reflected avian influenza worries, effect of media reports, and sense of vulnerability. Factor 5, 'feel protected' (6% of variance), items reflected reassurance from media reports, trust in government, and confidence in existing avian influenza control measures.

Models 1 to 3 were adjusted for sex, age, marital status, education, occupation, income, place of birth, years of residence in Hong Kong, and recent travel in mainland China. All models also included factors 1 to 5 plus attitudinal items not included in the factor scores.

Model 1 produced six independent predictors of buying live chickens: (1) travel: respondents reporting recent travel in mainland China were less likely to buy (adjusted odds ratio [AOR]=0.35; 95% CI, 0.1-0.9); (2) employment status: unemployed people were less likely to buy (AOR=0.18; 95% CI, 0.05-0.6); (3) traditional market practices (factor 2 score): persons supporting traditional markets were more likely to buy (AOR=1.2; 95% CI, 1.06-1.1); (4) protective practice (factor 3 score): persons reporting high protective practices were more likely to buy (AOR=1.2; 95% CI, 1.06-1.5); (5) willingness to change buying habits if other persons do the same (AOR=0.3; 95% CI, 0.1-0.8); and (6) belief that cooking food thoroughly is the best protection against bird flu (AOR=8.7; 95% CI, 1.6-46.7).

Model 2 estimated independent predictors of touching chickens when buying, using only respondents who reported buying live chickens themselves (n=451). Two variables independently predicted higher risk of touching: place of birth—persons born outside of Hong Kong—(AOR [China]=2.8; 95% CI, 1.4-5.4; AOR [elsewhere]=4.2; 95% CI, 1.4-12.5), and employment status—unemployment—(AOR=3.9; 95% CI, 1.2-12.1).

Model 3 identified adjusted independent predictors of risk perceptions for getting sick from buying live chickens. Older age lowered perceived risk (AOR [54 years of age]=0.3; 95% CI, 0.2-0.6; AOR [35-54 years of age]=0.5; 95% CI, 0.3-0.8 [reference, 18-34 years]), whereas worries about catching bird flu (AOR=2.9; 95% CI, 1.9-4.5), animal husbandry risk (Factor 1) [AOR=1.1; 95% CI, 1.04-1.14], protective practices (Factor 3) [AOR=1.1; 95% CI, 1.04-1.2], and avian influenza anxiety (Factor 4) [AOR=1.1; 95% CI, 1.0-1.2], all increased risk perception.

Discussion

Women are usually responsible for food shopping; shopping practices differ by gender, and reporting differences by gender have been found elsewhere.[5] The observed purchase (and therefore exposure) rate of 18.7 live chickens/household/year (38 370 343 purchases annually) matches government figures of about 38 325 000 live chickens purchased in 2004 in Hong Kong. This provides important independent validation of our data accuracy.

How much risk this exposure represents is difficult to accurately quantify. A highly conservative estimation assumes that genetic reassortment of human and avian influenza viruses can occur only on day 1 of a 5-day infectious period in a person with human influenza.[6] During the two 10-week human influenza seasons that occur annually in Hong Kong, sentinel data for influenza-like symptoms 1998 to 2004 indicate that peak population infection rates (pi) average 10% (±50% lower and upper bound estimates, ie 5-15%), giving $0.2 \times (4\,220\,738/52) \times 20 \times \text{pi} = 32\,467$ (16 233-48 700) episodes when persons on day 1 of a human influenza infection face exposure to live chickens. Wet markets amplify viral loads. Before the enactment in 2003 of wet market 'rest days', H5N1 isolates occurred in about 10% of chickens for sale in Hong Kong.[7] As all live chickens available in Hong Kong are vaccinated against avian influenza and the vaccine is presumed 90% effective, then only 1% (10% of 10% carrier rate) are potentially avian influenza infected, giving 325 (162-487) day 1 potential co-infection exposures when reassortment could occur, a rate of 0.0077% (0.0038-0.0115%). Influenza produces no symptoms for 24-48 hours after infection so shopping rates would be unaffected—assuming that 50% of persons shop on day 1 of infection reduces the figure by half to 162 (81-243) co-infection exposures annually. Among the 11% who touch the chickens, risk for avian influenza infection is likely to be greater. These estimates, though highly uncertain, quantify the potential risk involved.

Although one third of respondents perceived some risks from live chicken sales, risk magnitude seldom exceeded 60%, and peaks at 25% and 50% are partially artifactual.

Almost 50% indicated that their friends had expressed anxieties about avian influenza. Attributing greater concerns to others than to themselves reflects optimistic attribution bias, a protective response enabling expression of concern while preserving 'face'. Sickness anxieties reflected the fact that the markets and live chicken sales were perceived as health threats. Older persons, possibly due to past experience of buying live chickens, or past 'chicken plagues', viewed the present avian influenza outbreak as low risk. Hazard familiarity and experience can reduce associated risk perceptions. Respondents who reported higher anxiety and greater risk were no less likely to buy live chickens.

Raising population anxiety levels by warnings about disease produces only transient, inconsistent changes, and therefore appears to be ineffective as a means of reducing long-term high-risk behaviour. This is because (1) persons perceiving control over dubious 'hazards' seem to underestimate the associated risk, which reduces the likelihood of behaviour change; (2) persons who perceive little or no control over a threat adopt fatalistic responses continue with established behaviour, and direct coping efforts towards controlling emotions rather than risks; and (3) hazard exposure causes familiarity, thus reducing perceptions of risk. For these reasons, persons may dismiss the warnings as exaggerated or unrealistic. Once confidence in food safety is lost, recovery time may be protracted.

Conclusions

Perceptions of risk from buying live chickens were moderate, but sickness anxieties did not predict buying or touching habits. Buying was, importantly, strongly predicted by the belief that cooking is the best way to protect from avian influenza. This perception is an important message for health education groups seeking to increase preventive practices to control possible avian influenza outbreaks.

When planning for education programmes that aim to increase preventive practices to control possible avian influenza outbreaks, health education groups should remember that buying habits are strongly based on the erroneous belief that cooking is the best way to protect purchasers from avian influenza. Cooking protects from infection by eating, but not from infection through contact prior to eating.

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Clinical features and molecular epidemiology of coronavirus-HKU1–associated community-acquired pneumonia

Key Messages

1. Coronavirus (CoV)-HKU1 accounts for 2.4% of community-acquired pneumonia.
2. Clinical features alone cannot differentiate this entity from other community-acquired pneumonia.
3. Further studies are needed to understand the significance of CoV-HKU1 in upper respiratory tract infection and its potential to cause outbreaks of acute viral respiratory illnesses.

Introduction

No microbiological cause can be identified in a large proportion of patients with respiratory tract infections. Recently, we discovered a novel group 2 coronavirus—coronavirus HKU1 (CoV-HKU1)—from a patient with pneumonia.[1] We examined the prevalence of CoV-HKU1 in nasopharyngeal aspirate (NPA) samples from patients with community-acquired pneumonia during a 12-month period.

Aims and objectives

This study aimed to (1) define the clinical features of CoV-HKU1 infection, (2) understand the epidemiology of CoV-HKU1–associated pneumonia, (3) determine the molecular epidemiology and genotypes of the virus, and (4) assess the usefulness of diagnostic tests in identifying such infections.

Methods

Prospectively collected NPAs from patients with community-acquired pneumonia were sent to the clinical microbiology laboratories of four hospitals in Hong Kong during a 12-month period. Community-acquired pneumonia was defined as symptoms and signs consistent with an acute lower respiratory tract infection, together with new radiographic findings that develop before or within 48 hours of presentation. Once CoV-HKU1 was detected in NPAs, hospital records, laboratory results, and chest radiographs of the corresponding patients were analysed.

Possible risk factors associated with CoV-HKU1–associated pneumonia were determined using two age- and sex-matched controls per patient with CoV-HKU1–associated pneumonia that were randomly selected from patients with community-acquired pneumonia whose NPAs were negative for CoV-HKU1. Each set of controls was within 5 years in age (older or younger) and was admitted within 15 days before or after admission of the corresponding patient with CoV-HKU1–associated pneumonia. The hospital records, laboratory results, and chest radiographs of the controls were analysed.

Viral RNA was extracted from NPAs using the QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany).

RT-PCR of the pol gene of CoV-HKU1 was performed using CoV-HKU1–specific primers followed by DNA sequencing. A 453-bp fragment of the pol gene of CoV-HKU1 was amplified by RT-PCR using CoV-HKU1-specific primers (LPW1926 [5'-AAAGGATGTTGACAACCCTGTT-3'] and LPW1927 [5'-ATCATCATACTAAAATGCTTACA-3']) designed by multiple alignment of the nucleotide sequences of the pol genes of CoV-HKU1. Both strands of the PCR products were sequenced twice by use of an ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City [CA], US), using the two PCR primers. The sequences of the PCR products were compared with the sequences of the pol genes of CoV-HKU1 and those of other coronaviruses in the GenBank

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The ELISA-based immunoglobulin (Ig) G and IgM antibody tests were performed in accordance with our protocol. Each sample was tested in duplicate, and the mean absorbance for each serum sample was calculated.

The complete pol, S, and N genes of CoV-HKU1 from NPAs from nine of the 10 patients (from whom adequate amounts of RNA were available) were amplified and sequenced using the strategy described in our previous study. The nucleotide and deduced amino acid sequences of the pol, S, and N genes were compared with those of CoV-HKU1 and other group 2 coronaviruses. Phylogenetic tree construction was performed using the PileUp method with GrowTree (Accelrys Inc, San Diego [CA], US). Patient characteristics were compared between those with CoV-HKU1–associated pneumonia and those with non–CoV-HKU1–associated pneumonia, and between those who died of and those who survived CoV-HKU1–associated pneumonia. Fisher's exact test was used for categorical variables, and the Mann-Whitney *U* test was used for continuous variables. A P value of <0.05 was regarded as statistically significant.

Results

The NPAs from 10 (2.4%) of 418 patients with community-acquired pneumonia were positive for CoV-HKU1. All 10 cases occurred in winter and spring; nine of them were adults; and four had underlying diseases of the respiratory tract. In the six patients from whom serum samples were available, all had a four-fold change in IgG titre and/or presence of IgM against CoV-HKU1. The two patients who died had significantly lower haemoglobin levels, monocyte counts, albumin levels, and oxygen saturation levels on admission and had more extensive involvement visible on chest radiographs. Sequence analysis of the pol, S, and N genes revealed two genotypes of CoV-HKU1.

Discussion

Similar to HCoV-229E, HCoV-OC43, and HCoV-NL63, CoV-HKU1 was a human coronavirus that was endemic in humans. Similar to other human coronavirus infections, cases of CoV-HKU1–associated pneumonia occurred during winter and spring. Most patients with CoV-HKU1–associated pneumonia were old (80% were >65 years old) and had major underlying diseases, especially of the respiratory and cardiovascular systems.

Compared with SARS-CoV pneumonia, CoV-HKU1–associated pneumonia was a monophasic disease, and most patients had relatively mild symptoms localised to the respiratory tract and were therefore hospitalised only briefly. Although dyspnoea was present in 25% of patients with this pneumonia at presentation (compared to 20% in patients with SARS-CoV pneumonia), they often recovered quickly in contrast to those with SARS-CoV pneumonia who tended to deteriorate after 7-10 days.

Despite a relatively mild disease course in most patients, CoV-HKU1–associated pneumonia may be fatal in patients with low haemoglobin concentrations, monocyte counts, serum albumin levels, and oxygen saturation levels on admission and more extensive involvement on chest radiographs.

Conclusion

CoV-HKU1 is a cause of acute community-acquired pneumonia with winter seasonality. More studies should be conducted on this emerging cause of acute viral respiratory illness.

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AUTHOR INDEX

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