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Preliminary Findings of a Randomized Trial of Non-Pharmaceutical Interventions to Prevent Influenza Transmission in Households

Benjamin J. Cowling[1], Rita O. P. Fung[1], Calvin K. Y. Cheng[1], Vicky J. Fang[1], Kwok Hung Chan[2], Wing Hong Seto[3], Raymond Yung[4], Billy Chiu[5], Paco Lee[6], Timothy M. Uyeki[7], Peter M. Houck[8], J. S. Malik Peiris[2], Gabriel M. Leung[1]*

1 Department of Community Medicine and School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, **2** Department of Microbiology, The University of Hong Kong, Hong Kong, China, **3** Queen Mary Hospital, Hospital Authority, Hong Kong, China, **4** Centre for Health Protection, Department of Health, Government of the Hong Kong SAR, China, **5** Hong Kong Sanatorium and Hospital, Hong Kong, China, **6** St Paul's Hospital, Hong Kong, China, **7** Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **8** Division of Global Migration and Quarantine, National Center for Preparedness, Detection and Control of Infectious Diseases, Centers for Disease Control and Prevention, Seattle, Washington, United States of America

Abstract

Background: There are sparse data on whether non-pharmaceutical interventions can reduce the spread of influenza. We implemented a study of the feasibility and efficacy of face masks and hand hygiene to reduce influenza transmission among Hong Kong household members.

Methodology/Principal Findings: We conducted a cluster randomized controlled trial of households (composed of at least 3 members) where an index subject presented with influenza-like-illness of $<$48 hours duration. After influenza was confirmed in an index case by the QuickVue Influenza A+B rapid test, the household of the index subject was randomized to 1) control or 2) surgical face masks or 3) hand hygiene. Households were visited within 36 hours, and 3, 6 and 9 days later. Nose and throat swabs were collected from index subjects and all household contacts at each home visit and tested by viral culture. The primary outcome measure was laboratory culture confirmed influenza in a household contact; the secondary outcome was clinically diagnosed influenza (by self-reported symptoms). We randomized 198 households and completed follow up home visits in 128; the index cases in 122 of those households had laboratory-confirmed influenza. There were 21 household contacts with laboratory confirmed influenza corresponding to a secondary attack ratio of 6%. Clinical secondary attack ratios varied from 5% to 18% depending on case definitions. The laboratory-based or clinical secondary attack ratios did not significantly differ across the intervention arms. Adherence to interventions was variable.

Conclusions/Significance: The secondary attack ratios were lower than anticipated, and lower than reported in other countries, perhaps due to differing patterns of susceptibility, lack of significant antigenic drift in circulating influenza virus strains recently, and/or issues related to the symptomatic recruitment design. Lessons learnt from this pilot have informed changes for the main study in 2008.

Trial Registration: ClinicalTrials.gov NCT00425893, HKClinicalTrials.com HKCTR-365

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* E-mail: gmleung@hku.hk

Introduction

The specter of an influenza pandemic continues to threaten, with annual outbreaks of highly-pathogenic H5N1 in birds [1] and continued sporadic human H5N1 cases and clusters [2] with some reports that suggested limited, non sustained human-to-human transmission of H5N1 viruses [3,4]. If a pandemic virus strain were to emerge, pre-pandemic vaccines would be available to some populations although of unknown efficacy, but development and distribution of initial doses of influenza vaccine specifically made against the pandemic strain would not be available for at least 4–6 months [5]. Influenza antiviral medications would likely be in short supply in many regions, particularly in developing countries, and might have modest effectiveness against the pandemic strain, because of the emergence of antiviral resistance or other reasons [6]. Furthermore, few of these pharmaceutical measures can be applied at pandemic scale. Only non-pharmaceutical interventions [7–12] including use of face masks, improved hand hygiene, cough etiquette, social distancing measures, and travel restrictions would be available to the majority of the world's population. Interpandemic influenza is associated with thousands of deaths every year in Hong Kong [13] and likely hundreds of thousands worldwide every year [14,15], therefore simple personal protective measures could be beneficial during annual epidemics if found to be effective in reducing transmission, and as an adjunct to influenza vaccination.

We implemented a prospective cluster-randomized trial [16] to test whether two such non-pharmaceutical interventions can reduce transmission of interpandemic influenza in households.

Methods

The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Protocol S1 and Checklist S1.

Recruitment and follow-up of participants

From 30 first-contact outpatient clinics in both the private and public sectors across Hong Kong, we enrolled 944 Hong Kong residents aged at least 2 years, reporting at least two symptoms of influenza-like-illness (ILI) (such as fever $\geq$38°C, cough, sore throat, coryza, headache, malaise, chills, fatigue, etc.), and living in a household with at least two other individuals none of whom had reported ILI symptoms in the preceding 14 days. These index subjects provided nasal and throat swab (NTS) specimens which were combined and tested with the QuickVue Influenza A+B rapid diagnostic test (Quidel Corp, San Diego, CA) and those subjects with a positive result for influenza A or B were randomized and further followed up. For participants enrolled after June 1, 2007, those index subjects with a negative QuickVue result but a fever $\geq$38°C were also randomized and further followed up. Data on clinical signs and symptoms were collected for all subjects, and an additional NTS was collected for later confirmation of influenza infection by viral culture.

Following randomization a home visit was scheduled (to take place within 36 hours) to implement the intervention, collect baseline demographic data and NTS from all household members aged $\geq$2 years, and to provide and describe proper use of a free tympanic thermometer and the daily symptom record sheets. During the 9 days following the initial home visit, all household members were asked to keep symptom diaries, and three further home visits were scheduled at 3, 6 and 9 days after the baseline household visit to monitor adherence to interventions and to collect further NTS from all household members aged $\geq$2 years. At the final (day 9) home visit, the study nurse collected the symptom diaries and evaluated adherence to interventions by interview and by counting the number of surgical masks remaining or weighing the amount of soap and alcohol left in bottles and dispensers.

Ethics

All subjects aged 18 years and older gave written informed consent. Proxy written consent from parents or legal guardians was obtained for subjects aged 17 years and younger, with additional written assent from those aged 8 to 17 years. The study protocol was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster and was conducted in compliance with the Declaration of Helsinki [17].

Interventions

Our study compared three interventions. In the control arm, households received education about the importance of a healthy diet and lifestyle, both in terms of illness prevention (for household contacts) and symptom alleviation (for the index). Households in the face mask arm received the control intervention plus education about the potential efficacy of masks in reducing disease spread to household contacts if all parties wear masks, distribution of a box of 50 surgical masks (Tecnol – The Lite One, Kimberly Clark, Roswell, GA) for each household member (or a box of 75 paediatric masks for children aged 3–7 years), and demonstration of proper face-mask wearing and hygienic disposal. Index subjects and all household contacts were taught to wear masks as often as possible at home (except when eating or sleeping) and also when the index was with the household members outside of the household. Households in the hand hygiene group received the control intervention plus education about the potential efficacy of proper hand hygiene in reducing transmission, distribution of an automatic alcohol hand sanitizer (WHO recommended formulation II, liquid content with 75% isopropyl alcohol, Vickmans Labs Ltd., Hong Kong), liquid hand soap (Avalon organics glycerin hand soap, Petaluma, CA), individual small (125 ml) bottles of alcohol hand gel (Gellygen gel with 70% ethyl alcohol, Brymore SA, Italy), and demonstration of proper hand washing and hand antisepsis [18]. All household members including the index subject were taught to use the liquid soap in place of their regular soap after every washroom visit and in general when their hands were soiled or after sneezing or coughing, while they should use the alcohol hand sanitizer or hand rub when first returning home and immediately after touching any potentially contaminated surfaces. At the final home visit, households were reimbursed for their participation time with a supermarket voucher worth approximately US$20.

Objectives

The overall objective of the study was to quantify the efficacy of face masks and/or hand hygiene in reducing transmission of influenza to household contacts at the individual level. Specific objectives of this pilot study were to confirm the feasibility of the study design including the practicability of patient recruitment, randomization and follow-up, the appropriateness of the estimated sample size for a subsequent larger trial in terms of characteristics of local circulating influenza viruses and potential effect sizes, the applicability of the interventions and individual adherence with the interventions.

Outcomes

The primary outcome measure was the secondary attack ratio (SAR) at the individual level i.e. the proportion of household contacts of an index case who subsequently became ill with influenza. We evaluated the SAR using a laboratory definition (at least one follow-up NTS positive for influenza by viral culture or PCR) as the primary analysis, and three different clinical definitions of influenza as secondary analyses. The first definition of clinical influenza was fever $\geq$38°C or at least two of the following symptoms: headache, coryza, sore throat, aches or pains in muscles or joints, cough, or fatigue. The second definition was at least two of the following signs and symptoms: fever $\geq$37.8°C, cough, headache, sore throat, aches or pains in muscles or joints [19]. The third definition was the standard WHO/CDC influenza-like illness definition: fever $\geq$37.8°C plus cough or sore throat [20]. A secondary outcome measure was the secondary attack rate (SAR) at the household (cluster) level i.e. the proportion of households with one or more secondary case.

Sample size

We estimated that we would require 51 households (average size 3.8) in the control arm to allow determination of a secondary attack ratio of approximately 24% [21] to within +/−7%. Allowing for potential dropout, we therefore planned to recruit at least 60 households in the control arm, and a further 25–30 households to each of the face mask and hand hygiene arms to evaluate the feasibility of the interventions and allow a preliminary albeit imprecise estimate of efficacy. This pilot study was not powered to detect small or moderate efficacies of the interventions with statistical significance. We did not specify any early stopping rules or interim analyses.

Randomization

Randomization lists were prepared by a biostatistician (B.J.C.). Eligible study participants were randomly allocated to three groups. The first 100 households were randomized in the ratio 2:1:1 and subsequent households were randomized in the ratio 8:1:1 using a random number generator (R software). The rationale for changing the randomization ratio was to allow us to gather maximum information about the natural characteristics of influenza transmission in households in the absence of control measures, after evaluating the feasibility of each of the interventions in at least 25 households. Interventions were assigned to households by the study manager (R.O.P.F.) based on the randomization sequence. The allocation to specific intervention arms was concealed to recruiting doctors/clinics throughout.

Blinding

Participants and those administering interventions were not blinded to the interventions, but participants were not informed of the specific nature of the other interventions applied to other participating households.

Laboratory methods

Nasal swabs were collected by inserting and rotating a sterile swab (Collection swab; EUROTUBO, Madrid, Spain) into the anterior nares. Throat swabs were collected by rubbing a second sterile swab against the tonsillar fossa. Both swabs were snapped off into a tube containing viral transport medium (5% bovine serum albumin in Earle's balanced salt solution with antibiotic). At recruitment, additional nose and throat swabs were collected using sterile foam swabs and then combined and tested by the QuickVue Influenza A+B rapid diagnostic test.

Specimens collected from index subjects at recruitment were stored in a 2–8°C refrigerator (overnight, if required). Specimens collected during home visits were stored in a cool box with at least two icepacks immediately after collection. Before the end of the day of a home visit, study nurses took samples to the nearest collection point for storage in a 2–8°C refrigerator (overnight, if required) or directly to the central testing laboratory. Samples stored at 2–8°C were delivered to the central testing laboratory by courier in cool boxes en route. Samples were eluted and cryopreserved at −70°C immediately after receipt.

All clinical specimens were cultured on Madin-Darby canine kidney cells with exogenous trypsin (2 ug/ml) added. In households which were successfully followed up with home visits, the clinical specimens collected from index subjects at the recruiting clinic and during the first home visit were additionally tested by reverse transcription polymerase chain reaction (RT-PCR) for influenza A and B viruses if both specimens were negative by viral culture. For household contacts who reported symptoms during the follow-up but whose corresponding clinical specimens (collected within +/−2 days of self-reported fever or other respiratory symptoms) were negative by viral culture, those specimens were additionally tested for influenza A and B by RT-PCR. Additional technical details of the laboratory procedures employed in viral culture and RT-PCR testing are given in Text S1.

Statistical methods

To evaluate the SAR and to compare between groups we used exact binomial 95% confidence intervals, and χ^2 tests and multivariable logistic regression models adjusting for potential within-household correlation [22,23], with a 5% type I error rate. We estimated the intra-cluster correlation coefficient from the mean squared errors in the SAR between and within households [22]. All analyses were by intention-to-treat. We evaluated the three definitions of clinical influenza described above using receiver operating characteristic (ROC) analysis to determine the clinical definition that corresponds most closely to the laboratory outcome measure [24]. All analyses were conducted in R version 2.4.1 [25].

Results

Nine hundred and forty-four subjects were initially recruited to the study between February 24 and September 14, 2007. Figure 1 shows the progress of subjects and household contacts through the study. Overall, and in each intervention arm, the median household size was 4. Both the recruitment rate of subjects and the percentage of positive rapid influenza test results among recruited subjects increased in line with other measures of influenza activity including sentinel outpatient visits and laboratory isolations in mainly inpatient specimens during the periods of peak influenza activity in February and June (Figure S1). Of the 944 recruited subjects, 198 met the criteria for randomization and further follow-up. In a protocol deviation we randomized 9 subjects who had symptoms for (slightly) more than 48 hours; these 9 subjects were retained in the analyses.

Baseline data

Characteristics of the 198 subjects are shown in Table 1 according to intervention arm. In general the groups were well-matched. After randomization 70 (35%) of the households declined any home visits or could not be contacted after numerous repeated attempts. Proportionally more of these dropouts were in households where the index was a young adult, whereas there were few dropouts when the index subject was a child. Dropout was higher in households of index subjects who had a negative result on the rapid influenza test (25/44, 57%) compared to those who had a positive result (45/154, 29%).

We implemented the interventions in the remaining 128 households, and 127 (99%) were successfully followed for all four home visits; one household completed three home visits. (Table 1) The median household sizes were 4 in all intervention arms. We were typically able to apply the intervention within 1–2 days of symptom onset in the index case (Figure 2). Delays between symptom onset and intervention did not significantly differ between study arms (data not shown).

Numbers analyzed

Influenza could not be confirmed by viral culture or RT-PCR in the index subjects in 6 of the 128 households; therefore we only retained 122 households for analysis of crude SARs. Five household contacts had missing data on age, and these were further excluded for the multivariable regression analyses.

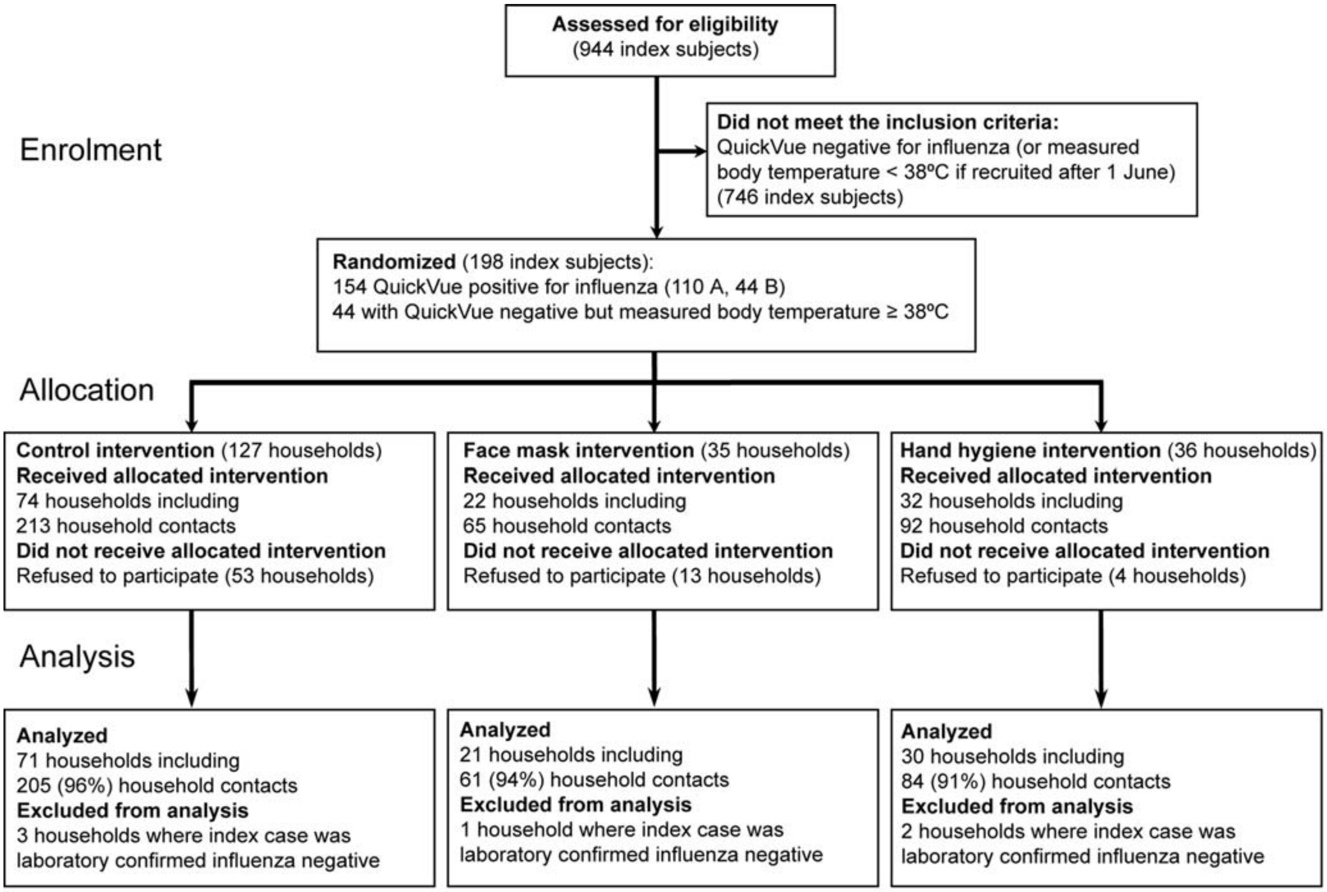

Figure 1. Flow of subjects through the study.
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Main outcomes

The overall laboratory-confirmed SAR was 6.0% (95% confidence interval 3.8%–9.0%) while the clinical SARs were 18%, 11% and 5% according to the three alternative definitions, respectively, with little difference between intervention arms (Table 2). The within-household correlation was 0.18 for the laboratory-confirmed SAR and varied from −0.05 to 0.01 for the various clinical definitions of influenza; chi-squared tests for differences in SARs between intervention arms were adjusted for these correlations (Table 2). The SARs were similar when stratified by the delay between onset of symptoms in the index case and application of the intervention (Table 2). Overall, 17/122 (14%) households had one or more laboratory-confirmed secondary case, while 44 (36%), 29 (24%) and 14 (11%) had one or more clinical secondary cases according to the three definitions above, respectively. SARs were similar when stratifying by influenza A or B infection in the index case (data not shown).

Table 3 shows the odds ratios of secondary infection in a household contact by intervention arm, adjusted for age, sex, influenza vaccination history and the age and sex of the corresponding index subject. Results were similar when stratified by the delay between symptom onset and application of the intervention (data not shown).

Ancillary analyses

A total of 24 index subjects were prescribed antivirals: 12 oseltamivir and 12 amantadine. By excluding these 24 households, the overall laboratory and clinical secondary attack ratios increased to 6.4% and 20%, 12% and 5% respectively, while the adjusted odds ratios of the intervention effects were similar (data not shown). Only three laboratory-confirmed secondary cases (4.5%) were observed in the 67 household contacts of the 24 index cases prescribed antivirals.

The 21 laboratory-confirmed secondary cases recorded a variety of clinical symptoms and 4 (19%) secondary cases were asymptomatic; all 4 asymptomatic cases were confirmed by viral culture. Of the three case definitions of clinical influenza, the second definition (based on [19]) had slightly higher discriminatory ability, with area under ROC curve 0.74, compared to the gold standard of laboratory outcome, whereas our original per protocol definition and the CDC definition had lower areas under the curve since the former was less specific while the latter was more specific but much less sensitive compared to laboratory-confirmed influenza (Appendix Table S1).

In terms of adherence, 45% (21%) of index subjects (household contacts) in the face mask arm reported wearing a mask often or always during the follow-up period, compared to 30% (1%) and 28% (4%) in the control and hand hygiene arms, respectively. The higher reported compliance in index subjects in the face mask group compared to household contacts was validated when at the final home visits the index subjects had used a median of 12 masks (inter-quartile range, IQR: 6, 18) whereas household contacts had only used a median of 6 (IQR: 1, 20); these include the mask worn and then disposed of by each individual as part of the demonstration and teaching during the initial home visit. A total of 63% (41%) of index subjects

Table 1. Characteristics of 198 randomized index subjects by intervention arm; the 128 index subjects successfully followed with home visits and their 370 household contacts.

	Control		**Face mask**		**Hand hygiene**	
Index subjects	**Randomized (n = 127)**	**Followed up (n = 74)**	**Randomized (n = 35)**	**Followed up (n = 22)**	**Randomized (n = 36)**	**Followed up (n = 32)**
Age group (%)						
2–15 years	48 (38%)	33 (45%)	12 (34%)	9 (41%)	13 (36%)	12 (38%)
16–30 years	23 (18%)	10 (14%)	7 (20%)	3 (14%)	7 (19%)	6 (19%)
31–50 years	32 (25%)	17 (23%)	11 (31%)	6 (27%)	10 (28%)	10 (31%)
50+ years	24 (19%)	14 (19%)	5 (14%)	4 (18%)	6 (17%)	4 (12%)
No. (%) men	60 (47%)	32 (43%)	16 (46%)	12 (55%)	14 (39%)	12 (38%)
Symptoms (%)						
Cough	99 (78%)	62 (84%)	24 (69%)	13 (59%)	33 (92%)	29 (91%)
Runny nose	98 (77%)	61 (82%)	28 (80%)	16 (73%)	28 (78%)	26 (81%)
Fatigue / tiredness	96 (76%)	56 (76%)	26 (74%)	16 (73%)	29 (81%)	25 (78%)
Fever (body temperature≥38°C)	94 (74%)	54 (73%)	25 (71%)	17 (77%)	29 (81%)	27 (84%)
Headache	80 (63%)	40 (54%)	29 (83%)	18 (82%)	22 (61%)	19 (59%)
Sore throat	69 (54%)	37 (50%)	23 (66%)	13 (59%)	22 (61%)	19 (59%)
Aches / pains in muscles or joints	62 (49%)	34 (46%)	18 (51%)	9 (41%)	18 (50%)	16 (50%)
Onset to randomization interval (%)						
0–24 hours	86 (68%)	48 (65%)	21 (60%)	14 (64%)	25 (69%)	22 (69%)
24–48 hours	35 (28%)	22 (30%)	12 (34%)	8 (36%)	7 (19%)	7 (22%)
48+ hours	5 (4%)	4 (5%)	1 (3%)	0 (0%)	3 (8%)	3 (9%)
Household contacts		(n = 213)		(n = 65)		(n = 92)
Age group (%)						
0–15 years	– –	32 (15%)	– –	11 (17%)	– –	14 (15%)
16–30 years	– –	43 (20%)	– –	13 (20%)	– –	17 (18%)
31–50 years	– –	92 (43%)	– –	28 (43%)	– –	35 (38%)
50+ years	– –	43 (20%)	– –	12 (18%)	– –	25 (27%)
No. (%) men	– –	83 (39%)	– –	26 (40%)	– –	37 (40%)
Influenza vaccination in the previous 12 months	– –	29 (14%)	– –	3 (1%)	– –	12 (6%)

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(household contacts) in the hand hygiene arm reported washing their hands often or always after sneezing, coughing or cleaning their nose compared to 31% (27%) and 63% (47%) in the control and face mask arms. In the hand hygiene group, households used a median of 56 g (IQR: 27 g, 93 g) of alcohol from the automatic sanitizer, and a median of 88 g (IQR: 63 g, 149 g) of liquid hand soap, while regarding the individual bottles of alcohol hand rub index subjects used a median of 7 g (IQR: 2 g, 13 g) and household contacts used a median of 5 g (IQR: 1 g, 12 g).

Adverse events

There were no reported adverse events, including allergic reactions to the intervention measures or other conditions requiring medical attention.

Discussion

If an influenza pandemic emerges, the likely limited supply of antivirals and vaccines will mean that non-pharmaceutical interventions have a major role to play in mitigating disease spread [11,12]. While conventional wisdom proposes that hand hygiene [8], and perhaps surgical masks [26], could be effective measures to reduce household transmission of influenza, all available data have so far been derived from at best observational settings and mostly based on anecdotal evidence rather than controlled trials [7,8,27]. Our study is the first reported community-based randomized trial of these interventions specifically against influenza, with laboratory-confirmed outcomes.

Strengths of our study design include the randomized allocation of interventions, the laboratory-based outcome measures, and our demonstrated ability to observe secondary infections with the implied potential to detect reduction in secondary attack ratios. Whereas the present study was not powered to assess the relative efficacy of the interventions, it has proved successful in demonstrating the feasibility of our study design and the local characteristics of influenza transmission. The present findings have facilitated the planning of a subsequent larger study, described in more detail in Protocol S1.

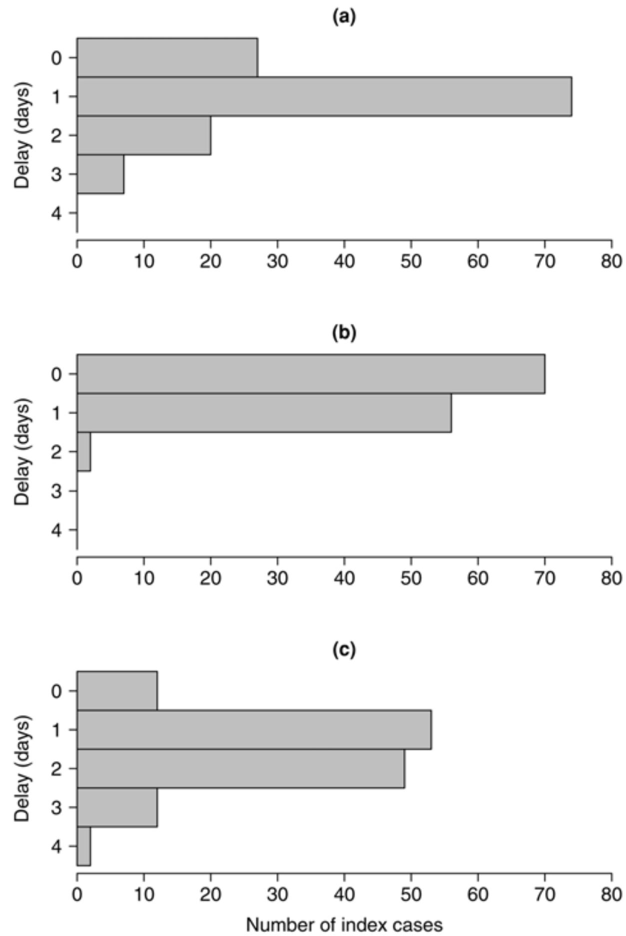

Figure 2. Delays between index case symptom onset, randomization, and intervention in 128 households. Time intervals a) from symptom onset in the index subject to randomization; b) from randomization to application of the intervention; c) from symptom onset to application of the intervention.
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Although we found little effect of the interventions in preventing household transmission, our study was underpowered. Nevertheless, our point estimates are close to null, strongly suggesting true equipoise until a definitive randomized trial with sufficient power (i.e. a much larger sample size) rigorously tests the relative efficacy of these interventions. A larger study will also allow us to explore in more detail the transmission dynamics of influenza in households including finer age stratifications and transmission within and between different age groups, which was not possible in the current study.

We observed generally low adherence to interventions. More than one in four household contacts in the face mask group did not wear a surgical mask at all during the follow-up period. Adherence to the face mask intervention was higher in the index subjects, likely due to their intention to reduce the probability of infecting other household members and possibly because of the recent memory of SARS in 2003, during which the majority (76%) of the general public reported that they wore face masks in public, and most engaged in numerous protective practices [28,29]. However more than one in four index cases in the control and hand hygiene intervention arms reported wearing masks at home of their own accord, thereby contaminating this intervention.

While self-reported hand-hygiene practices were similar across the three groups, we note that contamination of this intervention may be lower firstly because the control and face mask group did not receive the education component on proper hand hygiene, secondly because those groups did not receive the alcohol sanitizer and hand rub. Overall, adherence to the hand hygiene intervention in terms of soap and alcohol use appeared low when benchmarked against rates recommended in health care settings. However we note that a previous randomized community study found that 38% of households used more than 57 g of alcohol hand sanitizer during a 2-week period [30], whereas more than 50% of the households in our study used more than 56 g in 10 days.

Overall, the SAR was lower than we had expected. Only 6% of household contacts developed laboratory-confirmed influenza, whereas 5%–18% of contacts developed clinical influenza, depending on case definitions. This is in contrast to previous studies in France [21], Seattle [31] and other places [19], where SARs were approximately 25% (laboratory-confirmed influenza in the latter two studies). There could be a number of reasons for this. First, there has not been significant antigenic drift in the predominant circulating strains of influenza viruses in recent years, potentially resulting in higher levels of pre-existing immunity among our study population. Secondly, our inclusion criteria specified that an index subject should be the only member of their household to be suffering from ILI, and no other household contacts should have experienced ILI in the past 14 days, to ensure that the index is a true index within the household. However, the latter condition may have biased our recruitment towards households where some members were already immune from infection, since among households where all contacts were susceptible there might be a greater possibility of secondary cases being observed prior to the index case presenting to their primary care provider 1–2 days after symptom onset (Figure 2a). However the French study used similar inclusion criteria and found a much higher SAR [21]. Antiviral prescriptions for index subjects followed with home visits appears to affect transmission as would be expected [19], where there was a relative reduction in the SAR of approximately 30% albeit based on a limited sample size. Vaccination of household contacts might also have reduced the risk of secondary infection (Table 3). Finally, environmental or behavioral differences could lead to differing secondary transmission rates in our study, for example differences in use of air conditioning, high background use of face masks, or differences in the amount of time spent with family members at home. We did not collect the relevant data in the present study however; future studies should consider these externalities. Results from other settings with a similar design would be helpful in assessing, at least qualitatively, these respective effects.

The variability in clinical SARs depending on the choice of case definition has been noted in previous studies [21,32,33]. Influenza infection is associated with a wide spectrum of symptoms and severities, and in our study 4 (19%) of the 21 laboratory-confirmed secondary cases were asymptomatic. On the other hand, only 10 (48%) of laboratory-confirmed secondary cases reported fever $\geq 38^{\circ}C$. With such a range of symptoms caused by influenza, and when infections with other circulating upper respiratory viruses cause similar symptoms, collectively referred to as influenza-like-illnesses, it is difficult to find a single case definition which is highly sensitive and also highly specific for influenza virus. With a small sample size it is not possible here to derive clinical prediction rules [33–38], however we compared three alternative case definitions and

Table 2. Secondary attack ratios of laboratory-confirmed influenza and clinical influenza in the contacts of 122 analyzed households, by intervention arm.

Interval between symptom onset and intervention		Secondary attack ratio (95% CI*)						*p*-value†
		Control		Face mask		Hand hygiene		
		(n = 205)		(n = 61)		(n = 84)		
Any	Laboratory confirmed influenza	0.06	(0.03, 0.10)	0.07	(0.02, 0.16)	0.06	(0.02, 0.13)	0.99
	Clinical influenza definition 1‡	0.18	(0.13, 0.24)	0.18	(0.09, 0.30)	0.18	(0.10, 0.28)	1.00
	Clinical influenza definition 2‡	0.11	(0.07, 0.16)	0.10	(0.04, 0.20)	0.11	(0.05, 0.19)	0.97
	Clinical influenza definition 3‡	0.04	(0.02, 0.08)	0.08	(0.03, 0.18)	0.04	(0.01, 0.10)	0.52
		(n = 110)		(n = 32)		(n = 41)		
≤36 hours	Laboratory confirmed influenza	0.06	(0.03, 0.13)	0.12	(0.04, 0.29)	0.10	(0.03, 0.23)	0.69
	Clinical influenza definition 1‡	0.17	(0.11, 0.26)	0.25	(0.11, 0.43)	0.17	(0.07, 0.32)	0.76
	Clinical influenza definition 2‡	0.11	(0.06, 0.18)	0.09	(0.02, 0.25)	0.10	(0.03, 0.23)	0.98
	Clinical influenza definition 3‡	0.04	(0.01, 0.09)	0.09	(0.02, 0.25)	0.05	(0.01, 0.17)	0.44
		(n = 95)		(n = 29)		(n = 43)		
>36 hours	Laboratory confirmed influenza	0.05	(0.02, 0.12)	0.00	(0.00, 0.12)	0.01	(0.00, 0.12)	0.30
	Clinical influenza definition 1‡	0.19	(0.12, 0.28)	0.10	(0.02, 0.27)	0.19	(0.08, 0.33)	0.71
	Clinical influenza definition 2‡	0.12	(0.06, 0.20)	0.10	(0.02, 0.27)	0.12	(0.04, 0.25)	0.99
	Clinical influenza definition 3‡	0.05	(0.02, 0.12)	0.07	(0.01, 0.23)	0.02	(0.00, 0.12)	0.79

*Confidence intervals were calculated by the exact binomial method, not accounting for within-household correlation, and the resulting intervals may therefore slightly underestimate the uncertainty about the SARs.
†By Pearson chi-square test adjusted for within-household correlation.
‡Clinical influenza definition 1 is fever≥38°C or at least 2 of headache, runny nose, sore throat, aches or pains in muscles or joints, cough, or fatigue. Clinical influenza definition 2 is at least 2 of fever≥37.8°C, cough, headache, sore throat, aches or pains in muscles or joints. Clinical influenza definition 3 is the standard CDC classification of fever≥37.8°C plus cough or sore throat.
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found that the most predictive (with highest area under the ROC curve) was at least two of the following signs and symptoms: fever ≥37.8°C, cough, headache, sore throat, aches or pains in muscles or joints (Appendix Table S1). While the clinical SARs rely on self-reported symptoms, the diaries were checked for completeness and accuracy by trained nurses during home visits every 3 days. The proportion of asymptomatic infection in our study was lower than might have been expected (i.e. closer to 50%) based on earlier studies with paired serology [31], perhaps suggesting that we might have missed some infections when assessing the secondary outcomes of clinical influenza. The corollary is that the true secondary infection rate might well have been higher than estimated (and estimable) by our SARs.

The dropout was higher than anticipated; all subjects were advised of the study requirements and gave informed consent before being recruited into the study (and tested by rapid influenza test without charge), but 35% of randomized subjects/households refused to allow any home visits. These decisions were independent of the allocated intervention, since the interventions were only revealed during the first home visit. Dropout was higher among the group randomized with a negative result on the rapid diagnostic test (after June 1, 2008), perhaps because subjects interpreted their negative result as indicating they did not have influenza thus did not require follow-up. A negative rapid test result does not rule out influenza virus infection [39], and we chose to randomize such subjects to allow wider generalizability in terms of including index subjects with a likely greater range of influenza viral shedding profiles albeit with the limitation that some index subjects might have been infected with a different pathogen; in the latter case those households would be unnecessarily followed up since only households with index subjects with confirmed influenza (by viral culture or RT-PCR) were included in the final analyses. We found that dropout rates were lower when the index subject wasaged 15 years or younger (Table 1) perhaps because the accompanying parent would have also given immediate consent.

Other limitations of our study design include the potential bias from recruiting symptomatic subjects, resulting in three distinct effects. First, the use of a point-of-care test to detect influenza virus infection, ensuring that the majority of followed-up households will include an index case with laboratory-confirmed influenza (98% in our study), could also preferentially detect those potential recruits with higher viral shedding and subjects with lower levels of viral shedding would be more likely to receive a false negative rapid test results, and not be recruited. However we note that statistical power would be generally increased if index cases were more infectious since we might therefore observe more secondary transmission; the limitation here relates more to generalizability. Secondly, our design results in an unavoidable delay between onset of symptoms in the index subject and the application of the intervention (Figure 2c). If a significant amount of influenza transmission occurred prior to the intervention, we might have underestimated the efficacy of the non-pharmaceutical interventions or lacked the statistical power to find significant differences. In our analyses we investigated the SARs for those households where the intervention was applied within 36 hours of symptom onset but there was

Table 3. Factors affecting the laboratory-confirmed influenza and clinical influenza secondary attack ratios in the 350 household contacts.

	n	Laboratory-confirmed influenza		Clinical influenza*					
				Definition 1		Definition 2		Definition 3	
		OR†	95% CI for OR	OR†	95% CI for OR	OR†	95% CI for OR	OR†	95% CI for OR
Control group	202	1.00		1.00		1.00		1.00	
Face mask group	60	1.16	(0.31, 4.34)	0.88	(0.34, 2.27)	0.87	(0.30, 2.51)	2.00	(0.57, 7.02)
Hand hygiene group	83	1.07	(0.29, 4.00)	0.86	(0.39, 1.91)	0.88	(0.36, 2.14)	0.80	(0.22, 2.89)
Child (aged≤15)	54	1.00		1.00		1.00		1.00	
Adult (aged 16+)	291	1.75	(0.43, 7.16)	0.59	(0.31, 1.15)	1.40	(0.56, 3.53)	1.28	(0.36, 4.60)
Female	211	1.00		1.00		1.00		1.00	
Male	134	1.10	(0.52, 2.33)	0.87	(0.51, 1.47)	0.76	(0.39, 1.48)	0.99	(0.38, 2.58)
Not vaccinated	308	1.00		1.00		1.00		1.00	
Vaccinated in past 1 year	37	0.46	(0.07, 2.98)	1.42	(0.72, 2.79)	1.30	(0.55, 3.08)	0.63	(0.10, 4.07)
Child (aged≤15) index	52	1.00		1.00		1.00		1.00	
Adult (aged 16+) index	70	0.51	(0.18, 1.43)	0.83	(0.42, 1.66)	0.82	(0.36, 1.87)	0.55	(0.16, 1.84)
Female index	68	1.00		1.00		1.00		1.00	
Male index	54	0.80	(0.30, 2.13)	0.95	(0.48, 1.88)	0.79	(0.35, 1.80)	1.44	(0.43, 4.85)

*Clinical influenza definition 1 is fever≥38°C or at least 2 of headache, runny nose, sore throat, aches or pains in muscles or joints, cough, or fatigue. Clinical influenza definition 2 is at least 2 of fever≥37.8°C, cough, headache, sore throat, aches or pains in muscles or joints. Clinical influenza definition 3 is the standard CDC classification of fever≥37.8°C plus cough or sore throat.

†OR = odds ratio.

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no indication of greater efficacy in this subgroup. Thirdly, there is the potential for recruited households to be biased towards including household contacts with pre-existing immunity, as discussed above. An alternative approach would have been to randomize a much larger cohort of initially uninfected households, who were then followed throughout an influenza season. However such a longitudinal study would require greater resources by several orders of magnitude than the one proposed here, due to the low attack rate of influenza.

In conclusion, there remains a serious deficit in the evidence base of the efficacy of non-pharmaceutical interventions. The US Centers for Disease Control and Prevention have awarded grants to study non pharmaceutical interventions in community settings [40], including this study. Other funded study designs include symptom-based recruitment (as in our study) and longitudinal studies of initially uninfected cohorts, in children and adults and in various settings including households, schools and student halls of residences. We eagerly anticipate that conclusive evidence will become available as these studies proceed in the coming months, finally allowing empirically-driven pandemic planning.

Supporting Information

Table S1 Performance of alternative definitions of clinical influenza versus the gold standard of laboratory-confirmed influenza infection in household contacts.
Found at: doi:10.1371/journal.pone.0002101.s001 (0.03 MB DOC)

Figure S1 Study recruitment versus local influenza activity. a) daily recruitment rate (patients per working day); b) Local sentinel surveillance data of influenza-like-illness (number of ILI consultations per 1000 consultations) by the Centre for Health Protection; c) rate of positive influenza isolations among specimens submitted to the WHO reference laboratory of Queen Mary Hospital, Hong Kong.
Found at: doi:10.1371/journal.pone.0002101.s002 (1.23 MB EPS)

Text S1 Appendix with additional details of laboratory procedures
Found at: doi:10.1371/journal.pone.0002101.s003 (0.02 MB PDF)

Protocol S1 Trial protocol
Found at: doi:10.1371/journal.pone.0002101.s004 (0.17 MB PDF)

Checklist S1 CONSORT checklist
Found at: doi:10.1371/journal.pone.0002101.s005 (0.03 MB PDF)

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Author Contributions

Conceived and designed the experiments: JP GL BC WS RY. Performed the experiments: JP KC BC RF CC. Analyzed the data: JP GL KC BC VF. Contributed reagents/materials/analysis tools: JP KC. Wrote the paper: JP GL KC TU BC RF CC VF WS RY BC PL PH.

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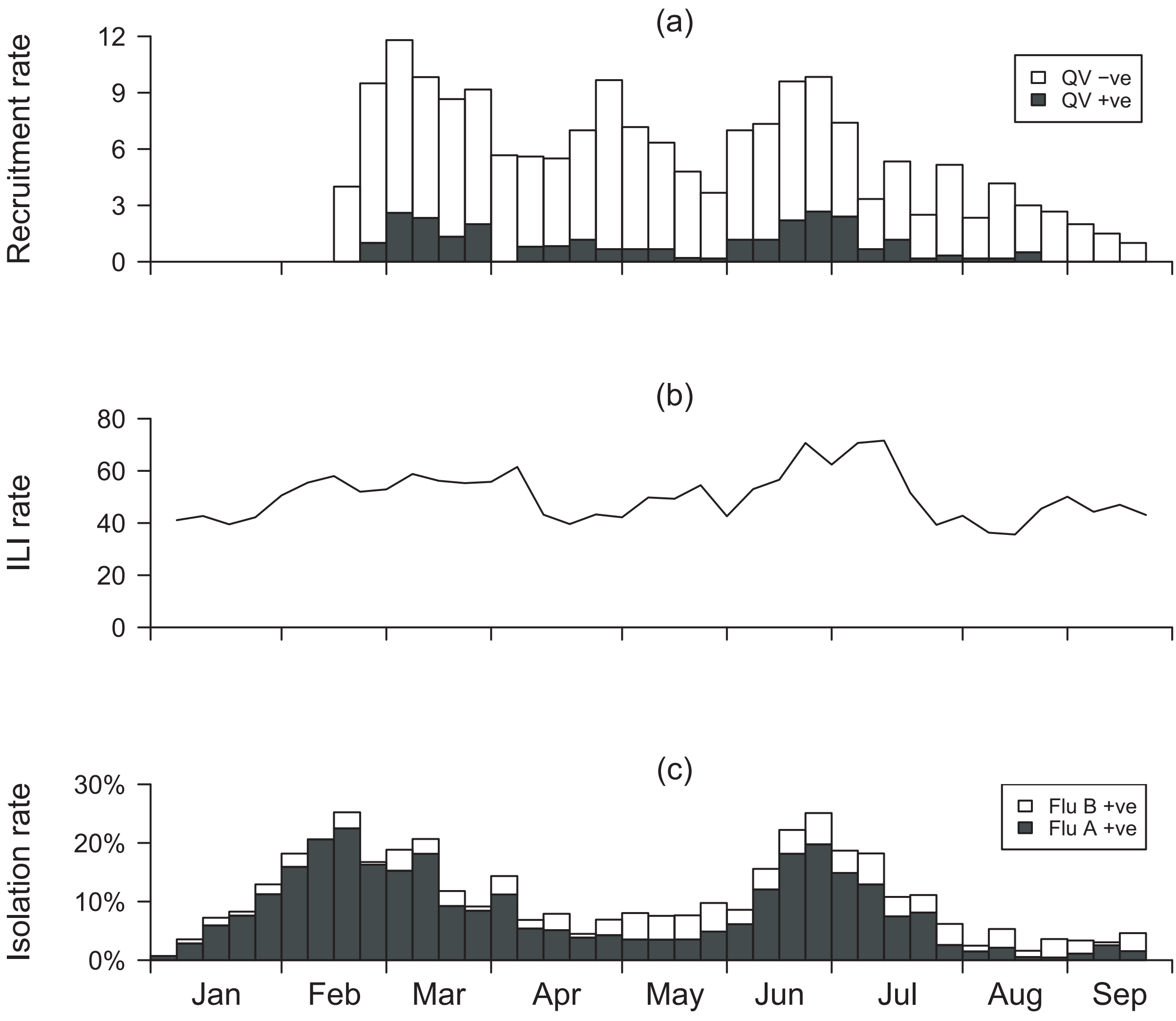
Table S1: Performance of alternative definitions of clinical influenza versus the gold standard of laboratory-confirmed influenza infection in household contacts.

Definition	Sensitivity	Specificity	Area under ROC (95% CI*)
Clinical influenza definition 1†	0.57	0.81	0.69 (0.59, 0.80)
Clinical influenza definition 2†	0.57	0.91	0.74 (0.62, 0.84)
Clinical influenza definition 3†	0.48	0.97	0.73 (0.61, 0.83)

*95% confidence interval for areas under ROC estimated by bootstrapping with 1000 resamples.

† Clinical influenza definition 1 is fever≥38°C or at least 2 of headache, runny nose, sore throat, aches or pains in muscles or joints, cough, or fatigue. Clinical influenza definition 2 is at least 2 of fever≥37.8°C, cough, headache, sore throat, aches or pains in muscles or joints. Clinical influenza definition 3 is the standard CDC classification of fever≥37.8°C plus cough or sore throat.


(a)
Recruitment rate
12
9
6
3
0
QV −ve
QV +ve
(b)
ILI rate
80
60
40
20
0
(c)
Isolation rate
30%
20%
10%
0%
Flu B +ve
Flu A +ve
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep

Laboratory Methods

Viral culture

Madin-Darby canine kidney (MDCK) cell monolayers in culture tubes were inoculated with 200 µl of the nasal swabs-virus transport medium suspension and the cells were maintained in serum-free minimum essential medium (MEM, Gibco, N.Y., USA) containing tosylsulfonyl phenylalanyl chloromethyl ketone-treated trypsin (2µg/ml) (Sigma, St. Louis, MO), and incubated at 33^{o}C for 7 days (MDCK cells). They were examined daily for cytopathic effect, and immunofluoresence was done on fixed cell smears when CPE appeared or at the end of the incubation period [1].

RT-PCR

Total nucleic acid was extracted from the specimens using NucliSens easyMAG extraction system (bioMerieux, Netherlands) according to manufacturer's instructions. Twelve µl of extracted nucleic acid was used to prepare cDNA by Invitrogen Superscript III kit with random primer as described previously [2] For influenza A or B virus, 2µl of cDNA was amplified in LightCycler with a total volume of 20µl reaction containing FastStart DNA Master SYBR Green I Mix reagent kit (Roche Diagnostics GmbH, Germany), 4.0mM $MgCl_2$ and 0.5µM of each primer. The forward primer (5'-CTTCTAACCGAGGTCGAAACG-3') and the reverse primer (5'-GGCATTTTGGACAAAKCGTCTA-3) were used for amplification corresponding to the M gene of influenza A [1]. Cycling conditions were as follows: an initial denaturation at 95ºC for 10 minutes, followed by 40 cycles of 95ºC for 10 seconds, 60ºC for 3 seconds, 72ºC for 12 seconds with ramp rates of 20ºC/second. For influenza B, forward (5'- GGGATATACGTAATGTGTTGT) and reverse (5'-GCACTGCCTGCTGTACACTT) primers was used to amplify a 489bp product corresponding to the nonstructural protein [3]. Cycling conditions were as follows: an initial denaturation at 95ºC for 10 minutes, followed by 45 cycles of 95ºC for 10 seconds, 55ºC for 5 seconds, 72ºC for 20 seconds with ramp rates of 20ºC/second. A series of dilutions were prepared to generate calibration curves and run in parallel with the test samples. At the end of the assay, PCR products were subjected to a melting curve analysis to determine the specificity of the assay.

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A Randomised Controlled Trial of Face Masks and Hand Hygiene in Reducing Influenza Transmission in Households

Protocol number GML001.5 (10 December 2007)

Investigators:

School of Public Health, The University of Hong Kong, Hong Kong
Professor G.M. Leung (**PI**)
Dr B.J. Cowling
Dr L.M. Ho

Department of Microbiology, The University of Hong Kong, Hong Kong
Professor J.S.M. Peiris
Dr K.H. Chan

Centre for Health Protection, Hong Kong
Dr R. Yung

Department of Microbiology, Queen Mary Hospital, Hong Kong
Dr W.H. Seto

Division of Global Migration and Quarantine, National Center for Preparedness, Detection and Control of Infectious Diseases, CDC, Washington, Seattle, USA
Dr P.M. Houck

Influenza Division, CDC, Atlanta, Georgia, USA
Dr T.M. Uyeki

St Paul's Hospital, Hong Kong
Dr. P. Lee

Hong Kong Sanatorium
Dr. B. Chiu

Pamela Youde Nethersole Eastern Hospital, Hong Kong
Dr Y.F. Choi

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1. Title

A randomised controlled trial of face masks and hand hygiene in reducing influenza transmission in households.

2. Background Information

Relevant definitions:
Index case: the first subject to be infected with influenza in a household.
Household contact: any person living in the same household as the index case.
Secondary attack ratio (SAR): the proportion of household contacts of an index case who subsequently become infected with influenza.
Hand washing: a process for the removal of soil and transient microorganisms from the hands [1].
Hand antisepsis: a process for the removal or destruction of transient microorganisms [1].

a) Name and description of the investigational products and interventions

The investigational products include surgical face masks, liquid hand soap and hypoallergenic waterless alcohol-based hand cleanser with emollient. The interventions will incorporate distribution of these investigational products and education on their proper use.

b) Relevant prior studies

There is currently concern about the possibility of an impending emerging influenza pandemic. In the event of such, a limited number of interventions would be available to reduce and control the spread of the disease and thus the resulting morbidity and mortality [2, 3]. Antiviral drugs could be used subject to availability, although their effectiveness against the novel pandemic strain is uncertain and resistance could develop quickly with large-scale use. Furthermore a vaccine specific to the pandemic strain would take optimistically, given current technology and production capacity, at least 6 months to develop and mass produce [4]. In addition to vaccination and targeted antiviral prophylaxis, other population-level social distancing measures such as school and workplace closures and travel restrictions are likely to be somewhat effective in reducing influenza transmission in the community [5, 6], but implementation on a prolonged basis and with repeated waves of the pandemic could be difficult. Household-based quarantine and isolation will likely be effective in mitigating the impact of a pandemic [5-7]. There is however considerable uncertainty about the efficacy of some non-pharmaceutical interventions at the personal level including face masks and hand hygiene. Our proposed study, to assess the efficacy of masks and hand-hygiene for influenza control, is a direct response to the World Health Organization’s recent call for urgent research on the efficacy of non-pharmaceutical public health interventions [3].

In addition to pandemic preparedness, knowledge about the efficacy of masks and hand hygiene would also be important for inter-pandemic influenza control. In western temperate and regional subtropical countries, influenza is a major source of morbidity and mortality during the seasonal periods of epidemic circulation [8, 9], and a number of measures are typically taken to try to reduce transmission in hospitals and elderly care homes [1]. However there are few data on the efficacy of such measures in the household, although household transmission is thought to be one of the most important settings for the community transmission of influenza [10].

Previous studies have tentatively suggested that prophylaxis with amantadine, rimantadine, and the neuraminidase inhibitors oseltamivir (Tamiflu®) and zanamivir (Relenza®) are effective in reducing influenza transmission in households [11, 12]. Vaccination is known to be an effective preventative measure [13] provided that the at least one of the vaccine strains closely matches the circulating strain [14].

Protective interventions at the personal level to reduce influenza transmission such as wearing masks and improving hand hygiene are often recommended [15] but few studies have investigated the efficacy of these measures outside nosocomial settings in a rigorous manner. Influenza is thought to be mainly transmitted through airborne droplet nuclei [16] but to a significant extent also spread by hand and surface transfer [17]. Some studies have suggested that transmission of upper respiratory tract infections was reduced after household-based hygiene interventions [18-20], while a recent case-control study has suggested that masks and hand-washing may have been effective in reducing the transmission of SARS in a hospital setting [21]. A recent population study has suggested that improved hygiene measures and decreased community mixing during the SARS outbreak in Hong Kong resulted in reduced incidence of respiratory viral infections [22].

The results of this study will have important implications for influenza prevention both in a pandemic and in interpandemic periods. Quantitative estimates of the efficacy of non-pharmaceutical interventions will inform resource allocation under pandemic preparedness plans.

c) Summary of known and potential risks and benefits

Wearing masks may diminish the rate of influenza transmission by reducing the amount of virus-containing droplet nuclei entering the surrounding area after leaving the mouth and nose of an infected subject. When worn by a non-infected subject in the presence of infected airborne droplets, surgical masks may reduce the amount of infected droplets inhaled and thereby reduce the chances of infection. However the degree to which a surgical mask can reduce airborne transmission is difficult to quantify given the lack of prior research in this area, and the expected benefit is uncertain. There are few apparent risks of wearing a mask, perhaps the greatest risk being that the mask engenders a feeling of overconfidence in the ability of the mask to prevent infection, leading to riskier activity (e.g. sitting closer to family members at mealtimes) than might have taken place if the mask were not worn, and thus an increased rather than decreased risk of influenza transmission.

Proper hand hygiene is thought to reduce community transmission of some viral infections including rhinoviruses and RSV [10], but the specific effect of hand hygiene on influenza has not been quantified. Again there are few apparent risks of proper hand hygiene, perhaps the greatest being the detrimental effects on skin of frequent hand washing, particularly with alcohol-based products although most of these contain emollients to buffer against excessive drying. To try to mitigate these effects as much as possible we will only supply an alcohol-based hand cleanser which includes emollients, and encourage study participants to be wary of skin irritation. The other very rare potential adverse consequence, as with all topical applications, is allergic reaction. We will use a hypoallergenic product and exclude participants who have a known allergy to alcohol or additive components of the alcohol handrub deployed.

d) Description of and justification for the route of administration and treatment period

The face masks and hand washing interventions will include an intensive counselling session to describe and demonstrate proper use of the respective hygiene aids.

Given that the index case could be symptomatic for a further 5 days, and asymptomatic incubation of the disease in household contact could take 1-2 days before symptoms appear, we propose that the hygiene measures should be maintained for at least 7 days.

e) Statement of proper conduct

The trial will be conducted in compliance with this protocol, GCP, and the applicable regulatory requirements.

f) Description of the population to be studied

The population studied are households in Hong Kong containing three or more individuals where at least one individual is suspected to be infected with influenza (ascertained either by meeting specific symptom criteria or by a positive result on a rapid diagnostic test, see 4(d)) and where no other household members have experienced symptoms of influenza-like-illness in the preceding two weeks.

3. Trial Objectives and Purpose

To quantify the efficacy of face masks and/or hand hygiene in reducing household transmission of influenza.

4. Trial Design

a) Primary endpoint

The primary outcome measure is the SAR i.e. the proportion of household contacts with laboratory-confirmed influenza during the study period (follow-up period of 10/7 days in pilot/main study). Inference will be made at the individual rather than household level, adjusting for the potential within-household correlation.

b) Trial design

The main study will follow a parallel design with three intervention arms (i.e. routine health education only, hand hygiene only, masks and hand hygiene). Households will be randomly assigned to one of the three interventions although intention-to-treat analysis will be at the individual level; therefore this is a cluster-randomised trial design. The pilot study will have three arms (mask only, hand hygiene only, routine health education only).

Subject recruitment will take place at selected government general outpatient clinics, group/managed practices, public hospital emergency rooms, private hospital outpatient departments, and private primary care clinics throughout Hong Kong, Kowloon and the New Territories.

For the pilot study we propose to recruit 500 individuals with ILI symptoms and apply the QuickVue rapid diagnostic test, so that we can follow-up a maximum of 200 index cases with a positive test result, and their households. We will stop recruiting as soon as we have 200 influenza-positive index cases, and we will stop recruiting after we have used 1,000 rapid diagnostic tests even if we have fewer than 200 influenza-positive index cases. Each household will be randomized to receive one of the three interventions, and all household contacts will be followed up. Details of the power calculation to justify this sample size are given below. Given an average household size of 3.8, a study of 200 households will involve the enrolment of a total of 760 individuals (200 index cases and 560 household contacts).

For the main study we propose to recruit approximately 6,000 individuals with symptoms of influenza-like illness (ILI) and follow-up an anticipated 1,200 index cases who meet specific criteria (in most cases a positive rapid diagnostic test result, or in some cases those meeting symptom based criteria – further described in 4(d)) and their households. Each household will be randomized to receive one of three interventions, and all household contacts will be followed up. Given an average household size of 3.8, a study of 900 households (from 1,200 randomized households after an anticipated 25% dropout) will involve the enrolment of a total of 3,420 individuals (900 index cases and 2,520 household contacts). Details of the power calculation to justify this sample size are given in 9(c) below.

c) Measures to minimise bias

A cluster-randomisation design has been chosen because simple individual randomisation of household contacts would almost certainly result in cross-contamination between family members who are assigned to different intervention arms. This cluster randomisation design requires a larger sample size to allow for potential within-household variability in SAR.

Given the nature of the interventions, participants will be un-blinded to the intervention received although they will be blinded to the nature of the other interventions. The same will apply to our research nurses who will be educating participating households on uses of assigned interventions and prevention of influenza transmission. Nurses will initially be randomly assigned to administering one of the three interventions and will receive training relevant to their assigned intervention only, hence avoiding potential cross-contamination by nurses.

When the details of a new index case are uploaded to the online database by fax to the trial manager, a unique identifier will be assigned. A pre-specified table of random numbers will be used to assign one of the three interventions to the household of the index case. Therefore the randomised intervention will be unknown to the doctor at or after the time of recruitment to minimise allocation and ascertainment biases respectively.

d) Criteria for further follow-up

In the majority of recruiting sites, the criteria for further household follow-up will be a positive result for influenza A or B using the QuickVue rapid diagnostic test on a nose and throat swab (Quidel Corp, San Diego, CA) which has a reported sensitivity of 79% and specificity of 92% for influenza A or B [23]. If the QuickVue test is positive and informed consent is obtained, the index case and their household will be further followed up with home visits as described above. We will calculate the sensitivity and specificity of the rapid diagnostic test in our study setting, using viral culture or PCR of a nose and throat swab as the gold standard.

In a small number of recruiting sites at defined periods during the pilot and main studies we will use a symptom-based criteria to determine eligibility for further follow-up; specifically we will enrol subjects and their households for further follow-up if they present with at least two of the following symptoms: fever≥37.8°C (≥38°C in the pilot study); cough; headache; sore throat; pain in muscles or joints [24].

In a small number of recruiting sites at defined periods during the main study we will use an alternative rapid diagnostic test, namely the HX Diagnostics Influenza A+B test (HX Diagnostics, San Francisco, CA) which is based on 3rd generation lateral flow immunoassay technology and may be more sensitive and specific than the QuickVue test. In this case, subjects with a positive result on the HX Diagnostics Influenza A+B test would be eligible for further follow up as described above. We will calculate the sensitivity and specificity of the rapid diagnostic test in our study setting, using viral culture or PCR of a nose and throat swab as the gold standard.

See also 7(c).

e) Trial interventions

See 6(a).

f) Home visits

Following randomization, an immediate home visit will be scheduled (to take place within at most 36 hours, and ideally within 12 hours) to implement the intervention. During the home visit by a trained nurse, the purpose of the study will be explained to all household contacts and their consent obtained. Consent for children aged 17 years or younger will be obtained from their parents. Assent will also be obtained for children aged between 7 through 17. The nurse will then collect details on household composition, risk perceptions, attitudes and beliefs on influenza (questionnaire Q2, appendix), and take a nose swab and a throat swab from each household contact, except for asymptomatic children under the age of 2. Due to concerns about difficulties of taking nose and throat swabs from infants, for household members who are under 2 years of age only information on clinical symptoms will therefore be collected unless they are symptomatic. For participants who are symptomatic, a nose and a throat swab will be collected regardless of their age. The swabs will later be tested to confirm the absence of influenza in any household contact at baseline. The nurse will provide and describe proper use of a free tympanic thermometer, and the daily symptom diaries. Finally, the nurse will administer the standardized intervention through intensive counselling, and demonstration of proper wearing of masks or hand washing.

Three and six days (±1 day) after the initial home visit, a trained nurse will revisit each household. During the visit, the nurse will take nose swabs and throat swabs from the index case and all household contacts, except for children under 2 years of age who are asymptomatic, and collect the symptom diaries (questionnaire Q3, appendix) from each household contact. During the final visit (day 6), the nurse will ask the household members to complete a final questionnaire (Q4, appendix) and assess their adherence to the interventions (questionnaire Q5, appendix).

g) Trial period

The 8-week pilot study will take place from January to April 2007. The exact starting and stopping dates of patient recruitment will not be fixed in advance. Recruitment will begin after the start of the annual influenza peak season (typically Feb/Mar) has been confirmed by the Department of Microbiology, HKU, and will continue until the prespecified sample size is reached. The 39-week main study will take place from January to September 2008.

h) Stopping rules

Individuals may at any time decide to stop participating if they wish, without prejudice or any adverse consequences. There are no formal rules for stopping the trial early.

i) Maintenance of trial treatment randomisation codes and procedures for breaking codes.

When the details of a new index case are uploaded to the online database, a unique identifier will be assigned. A table of random numbers will be generated by the trial statistician prior to the start of the trial, and this will be used to assign one of the three interventions to the household of the index case. Randomisation codes will not be used since for the first home visit it is necessary for the nurse to know which of the interventions has been assigned.

Randomisation codes will be masked from those assessing the outcomes.

k) The identification of any data to be recorded directly on the case record forms (i.e. no prior written or electronic record of data) and to be considered source data.

See questionnaires Q1-Q5 (appendix) for the source data that will be recorded in this study. Note that we will not access subjects' medical records.

l) Prevention of influenza in study nurses

Nurses will be offered influenza vaccination prior to the study.

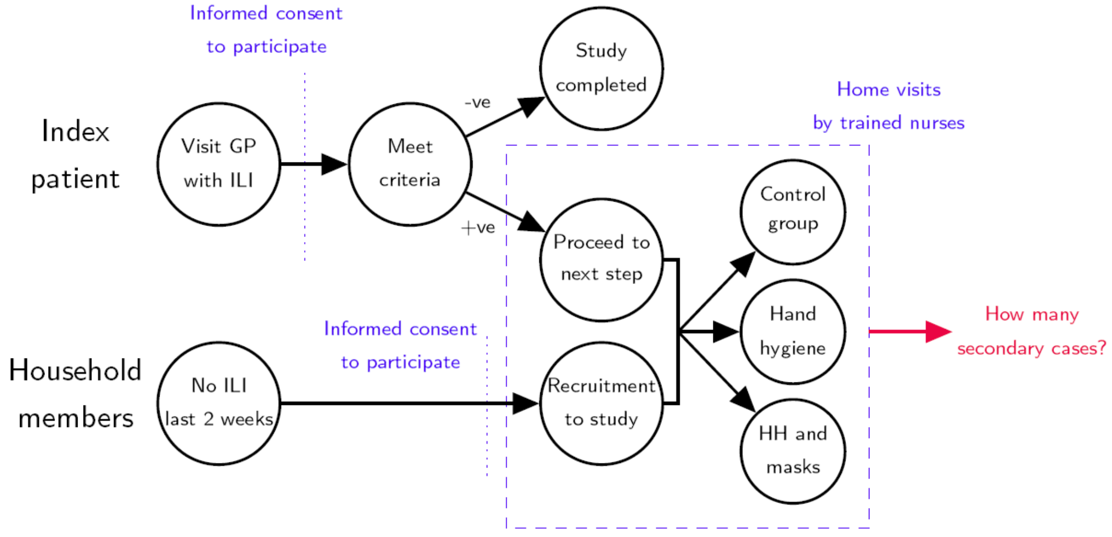
m) Incentives to participate

We will offer each participating household a HK$200 (US$25) supermarket voucher (HK$150 / US$20 in the pilot study).

n) Centralized collection and storage of specimens

Specimens collected in recruiting clinics will be stored in a 2-8°C refrigerator (overnight, if required). Specimens collected during home visits will be stored at room temperature or if necessary (i.e. in hot weather) in a cool box with at least 2 icepacks immediately after collection. Before the end of the day of a home visit, the study nurse will take any collected samples to the nearest recruitment clinic for storage in a 4°C refrigerator (overnight, if required) or directly to the Department of Microbiology, HKU. Samples stored at 2-8°C in recruiting clinics will be delivered to the Department of Microbiology, HKU as soon as possible, via courier and maintained at 2-8°C en route. Samples will be eluted and cryopreserved at minus 70°C at the destination.

o) Flow chart of main study design



The criteria for further follow-up are discussed in 4(d), in the majority of recruiting sites the criteria will be a positive result on the QuickVue Influenza A+B rapid diagnostic test.

p) Buccal swabs

During the final home visit in the main study we will request buccal swabs from all household members. The samples will be anonymised and then processed by a biotech company specialising in DNA extraction from human samples. These data will be studied at a later date to investigate possible genetic factors in influenza susceptibility or transmission.

5. Selection and Withdrawal of Subjects

a) Inclusion criteria for index cases and households

Inclusion criteria for index cases are as follows: (1) a Hong Kong resident; (2) reporting ILI symptoms including at least two of fever (recorded fever ≥38°C), cough; nasal congestion; sore throat; headache; runny nose and pains in muscles or joints [24] (3) onset of symptoms within the preceding 48 hours. If these conditions are satisfied, the subject will be approached to determine household eligibility to enrol in the study as below prior to further follow-up as described in 4(d) and 4(f).

Inclusion criteria for households are as follows: The household must contain at least three people including the index case and any domestic helpers, and where no household contacts have had ILI symptoms in the preceding two weeks.

In the main study (January to September 2008), if we are ahead of our recruitment target at any time in March or later, we will consider revising the inclusion criteria to include only subjects with onset of symptoms in the preceding 36 (or 24) hours, since we believe the interventions will be most effective if applied sooner after symptom onset. If implemented, this protocol change would be taken into account in the final analyses.

b) Subject exclusion criteria

There are no exclusion criteria.

c) Subject withdrawal criteria

The entire household will be withdrawn from the study if there is failure to obtain proper informed consent from any one or more individual household contacts for whatever reason. Informed consent will be sought from each individual household contact, or proxy consent from the parents of any individual under the age of 18. Assent will also be obtained for children age between 7 through 17. Households will be withdrawn from the trial if no home visit can be scheduled within 36 hours. Withdrawn households will be replaced to maintain the specified sample size. If one or more household contacts are not present during one of the home visits, we will attempt to reschedule a supplementary home visit to collect clinical specimens, and in the case where the initial home visit was missed we will request signed informed consent and apply interventions as necessary during the supplementary home visit.

In the main study, if a randomized household refuses to allow any home visits, we will request permission to contact them by telephone after 7 days to enquire how long the index case experience symptoms for, and whether any household members reported clinical influenza; if available, these data will allow a simple comparison of households who dropout with those who are successfully followed up.

6. Treatment of Subjects

a) Treatments administered

We will randomize households among three study arms, each of which will include intensive counselling during the first household visit. All recruited households will receive educational pamphlets specific to their assigned intervention arms in English or Chinese or both, as appropriate. The intervention arms are as follows:

(i) Control intervention (general health education):
This group will receive education about the importance of a healthy diet and lifestyle for boosting the immune system against influenza and other directly transmitted respiratory pathogens leading to ILI symptoms, both in terms of illness prevention and symptom alleviation.

(ii) Hand hygiene intervention:
This group will receive the control intervention (health education) plus education about the potential reduction in transmission of respiratory infections to household contacts if all parties maintain proper hand hygiene, and demonstration of proper hand washing and hand antisepsis. Each household member (including the index case) will receive a uniquely labelled 100ml bottle of hypoallergenic waterless alcohol-based hand rub for individual use only, and households will be provided with one 220ml bottle of antimicrobial Ivory liquid hand soap (Proctor & Gamble, Cincinnati, OH) for each washroom and kitchen sink.

(iii) Face mask and hand hygiene intervention:
This group will receive the control intervention plus the hand-hygiene intervention plus education about the potential reduction in transmission of acute directly transmitted respiratory infections to household contacts if all parties wear masks, distribution of 50 (75) surgical masks for each adult (child aged 3-7) household member, and demonstration of proper face-mask wearing.

b) Randomisation plan

The pilot study will include the first two arms and a third mask-only arm (i.e. the mask intervention but not the hand hygiene intervention). For the first 100 households, we will apply an unbalanced randomisation of 2:1:1 among arms (1), (2) and (3). For the subsequent households (up to a further 100 households) we will apply an unbalanced randomisation of 8:1:1 among arms (1), (2) and (3) to allow us to extract maximum information about the transmission dynamics of influenza in the absence of non-pharmaceutical control measures. If the maximum sample size of 200 households is reached, there will be approximately 130, 35 and 35 households in arms (1), (2) and (3) respectively. Following completion of the pilot study, information derived about the characteristics of influenza transmission will be invaluable not least in validating our sample size calculation for the main study.

The main study will randomise households equally among the three arms above, using a block randomisation structure with randomly permuted block sizes of 18, 24 and 30.

We will use separate randomisation tables for subjects recruited with different criteria (as described in 4(d)) to ensure the intervention groups are balanced; this is because the QuickVue test is likely to capture subjects with on average higher viral shedding than a symptom-based criteria.

c) Medications permitted and not permitted before and/or during the trial

There are no restrictions on the use of other medications during the trial period. However the use of antivirals, antibiotics and other Western and Chinese medicine to relieve ILI symptoms during the study period will be recorded by the visiting nurse in the nurse's assessment sheet at the first and last home visits (ie. Questionnaire Q2 and Q4 respectively, appendix).

d) Procedures for monitoring subject compliance

Self-reported use of hygiene measures including mask wearing and hand washing will be recorded in the symptom diaries (Q3) by each household contact and the index case. Overall use will be reported at the final home visit, and quantities of masks, alcohol and liquid hand soap used will be measured by the visiting nurse during the final household visit.

7. Assessment of Efficacy

a) Specification of the efficacy parameter

The primary outcome measure is the secondary attack ratio (SAR) which is the proportion of household contacts with laboratory-confirmed influenza during the study period (10/7 days after recruitment in the pilot/main studies). We will preferentially use the laboratory definition of influenza rather than the clinical definition, when available.

b) Methods and timing for assessing, recording and analysing of efficacy parameter

Household contacts will be confirmed to be influenza-free at the first household visit, within 36 hours of recruitment of the index case.

In the main study, clinical influenza is defined as the presence of at least two of the following symptoms: feverishness (we will strongly encourage household members to use the supplied thermometer to assess whether a fever is ≥38°C); cough; headache; sore throat; pain in muscles or joints (following [24]). In the pilot study (as per the initial protocol), clinical influenza is defined as the presence of feverishness, or at least two of the following symptoms: cough; sore throat; nasal congestion, rhinorrhoea, or sneezing; fatigue; headache; stiffness; myalgias.

In the main study during the follow-up period of 7 days after recruitment of the index case, the index case and all household contacts will be asked to maintain a daily record of their symptoms (questionnaire Q3, appendix) and their tympanic temperature. A nurse will visit the household on three occasions during follow-up, namely 3 and 6 days after the initial home visit (day 0) with a window period of ±1 day. During each visit the nurse will collect nose swabs and throat swabs from the index case and all household contacts. These will be cryopreserved at the Department of Microbiology HKU as soon as possible as decribed in 4(n). The nose swabs and throat swabs taken during the follow-up visits will provide independent confirmation of the presence or absence of influenza virus in all household contacts, and the duration of viral shedding in the index case. In the pilot study the follow-up period will be 10 days, with visits on days 0, 3, 6 and 9.

The primary endpoint of our study will compare the SAR in each of the intervention groups with the control intervention. We will use χ^2 tests and odds ratios adjusting for potential within-household correlation, with a 5% type I error rate.

c) Diagnosis of influenza in index case

The criteria for further follow-up of the index subject and their household were described in 4(d) above. In the majority of cases, the criteria for further follow-up will be a positive result on a rapid diagnostic case. In some recruiting clinics the criteria will be presentation with at least two of the following symptoms: fever≥37.8°C (≥38°C in the pilot study); cough; headache; sore throat; pain in muscles or joints. However we will only include households in the final analyses if the index subject is laboratory confirmed to have influenza infection. This laboratory confirmation will require a positive result for influenza A or B by viral culture or standard PCR of a nose and throat swab collected from the index case at the recruitment site, and/or during the first home visit.

d) Assessment of factors which may affect the rate of influenza transmission

During the first household visit, a responsible adult (usually the household head or a parent) will be asked to provide an overview of the composition of the household, and details on past illness history and influenza vaccinations (Q2, appendix). At the final household visit, the nurse will collect information (questionnaire Q4, appendix) on the overall self/proxy-reported compliance with the intervention, and on any medication taken during the follow-up period, by asking household members and also by personally checking how many masks remain unused, or how much soap or alcohol is left in the bottles and dispensers.

8. Assessment of Safety

a) Specification of safety parameters

There are no safety parameters in this trial.

9. Statistical Analysis

a) Statistical methods to be employed

The characteristics of households, index cases and household contacts in the three intervention groups will be compared and assessed for similarity with χ^2 tests, adjusting the comparison of household contacts for potential within-household correlation. In the primary analyses, households will only be included if the index case has laboratory-confirmed influenza infection – all other households will be dropped from the primary analysis. Furthermore, in the main study households will be dropped from the primary analysis if any of the household contacts are found to have laboratory-confirmed influenza infection at baseline although this will not be incorporated in analyses for the pilot study as per the original protocol. Therefore our results will not be biased by the potentially different transmission dynamics of other respiratory diseases compared to influenza A or B, or by the potential for more than one index case in a household when the interventions are applied.

The primary endpoint of our study will compare the SAR in each of the intervention groups with the placebo intervention (1). We will use χ^2 tests and odds ratios adjusting for potential within-household correlation [25], with a 5% type I error rate.

We will investigate the efficacy of the interventions on the SAR in multivariable logistic regression models with a generalized estimating equations approach to allow for potential within-household correlation [26]. Analysis will first be performed including only the effects of masks and hand hygiene. Further analyses will allow for the effects of potential confounders on the SAR. Confounders of the SAR that we will assess for each household contact include the age, gender, smoking status, chronic disease status, prior vaccination status, and additionally the age and gender of the corresponding index case. We will investigate the intervention effect in age/gender subgroups, although the statistical power for these analyses is unlikely to be high. We will further investigate the intervention effect in households where the intervention was applied sooner (with 36 hours) after symptom onset in the index case.

We will further investigate the intervention effects for influenza A and influenza B separately, although with likely lower incidence the statistical power for the latter may be low.

We will also assess the adherence of the index case and household contacts to the interventions, and conduct as-treated analyses of the primary outcome measure. We will conduct sensitivity analyses excluding households where the index case was prescribed antiviral medication, since onward transmission may be less likely in this scenario.

b) Planned secondary analyses

In further analyses, we will investigate the effect of the interventions on secondary outcomes listed below, and further adjust for the effect of possible confounders in multivariable logistic and proportional hazards regression models where appropriate.

1. The proportion of household contacts with clinical influenza, adjusting for the potential within-household correlation.
2. The proportion of households with one or more secondary case of influenza (laboratory definition used in preference to clinical definition where available).
3. The proportion of households with one or more secondary case of clinical influenza.

4. The time from intervention to first symptoms of clinical influenza among household contacts.

We will investigate the predictors of influenza infection and the factors affecting duration of symptoms. We will further examine the effect of environmental and lifestyle factors, and measures of risk perception on the disease course and onward transmission using regression models. We will examine the factors affecting adherence to interventions using regression models.

We will develop and apply novel modelling approaches to the analysis of the household infection data to estimate specific transmission parameters, building on previous research [27].

We will investigate the performance of the QuickVue Influenza A+B rapid diagnostic test (and the HX Diagnostics Influenza A+B test) by comparison with the gold standard of laboratory confirmed influenza by viral culture or PCR. We will further investigate the factors potentially affecting rapid diagnostic test performance, including age, gender, and time since symptom onset.

If funding is available, we will conduct further laboratory tests of collected samples to allow us to investigate the incidence and transmission dynamics of non-influenza respiratory viruses. Subjects' consent for these additional respiratory virus tests are provided on the current version and all previous versions of the informed consent forms.

If funding is available, we will apply quantitative PCR tests to nose and throat swabs collected from home visits, to investigate potential correlation between the degree of viral shedding and onward transmission, as well as the degree of viral shedding in any resulting secondary cases.

Finally, if funding is available, we will sequence the genome of influenza viruses detected in index cases and secondary cases to investigate genetic variability in the virus as well as the evolution rate between successive cases (and indeed whether secondary cases were truly infected by their corresponding household index, or from some other source).

c) Planned sample size

Pilot study: A simple calculation of the SAR is given by dividing the number of household contacts with influenza by the total number of household contacts. To estimate the anticipated SAR of 0.241 to within ±5% would require 283 household contacts. Given an average household size of 3.8 (i.e. 2.8 contacts per household) we would require at least 101 households in the placebo group; we propose to recruit 130 households in this arm to allow for some households being lost to follow-up. To test the randomization and intervention procedures we will recruit a further 35 households to the second and third study arms (masks and hand-hygiene). This will also enable a preliminary estimate of the efficacy of hand hygiene and masks although the power of the pilot study will be low to detect small or medium effect sizes with statistical significance. More importantly, the pilot study will allow an idea of the feasibility of these interventions, and coherence to them.

Main study: For the sample size calculation we require an estimate of the anticipated SAR (P), the degree of within-household correlation (ρ) in the SAR, the relative risk (r) that we would like to detect, and the relevant critical values of the standard normal distribution Z for a specified power (1-β) and type I error rate (α). For average household size m, the required number of individuals n in each intervention arm is given approximately by

$$n = \frac{\left(Z_{1-\alpha/2} + Z_{\beta}\right)^2 \left[P(1-P) + rP(1-rP)\right]\left[1+(m-1)\rho\right]}{(P-rP)^2},$$

and thus the number of required households is given by n/m [25].

A recent study of influenza transmission in household contacts in France found a SAR of 24.1%, and a within-household correlation of ρ=0.29 [28]. We will assume a reduced SAR of 20% to allow for the possibility of some transmission occurring prior to randomization and the likely inclusion of some index cases without influenza. A relative risk reduction of at least 30% is generally accepted to be clinically and epidemiologically important. We note that the efficacy of masks and hand-washing were estimated to give relative risk reductions of 90% and 75% respectively during a nosocomial outbreak of SARS, and while we doubt such high efficacy in the household setting we anticipate relative risk reductions of perhaps around 30%-50%, although there is no literature to guide us on such estimates (and hence the need for this trial!). The average household size in Hong Kong excluding houses with single or double occupancy is 3.8 (source: Hong Kong Thematic Household Survey 2002), therefore the average number of household contacts per index case would be m=2.8.

We would like to have at least 80% power to detect a 30% reduction (i.e. r=0.7) in the relative risk between intervention 2 (or intervention 3) and intervention 1 (anticipated P=0.2), with a 5% type I error rate. Using the formula given above we calculate that we would require the randomization of 840 household contacts into each arm of the study. Allowing for a 25% dropout rate following randomization, we would require the randomization of 840 household contacts into each study arm corresponding to a total study requirement of 2,520 household contacts in 900 households. Thus we will recruit a total of 3,420 individuals including 900 index cases with positive results on the QuickVue rapid diagnostic test, and 2,520 household contacts. The specified sample size would also have high statistical power to detect larger relative risk reductions if the observed secondary attack ratio were lower (Table 1).

Table 1: Power of proposed study (n= 840 in each arm after allowing for a 25% dropout) to detect statistically significant intervention effects of various sizes and for various secondary attack ratios, with 5% type I error.

Intervention effect (as a relative risk reduction)	Secondary attack ratio 0.25	0.20	0.15	0.10
20%	0.51	0.41	0.31	0.21
30%	0.86	0.76	0.61	0.43
40%	0.99	0.95	0.87	0.69
50%	>0.99	>0.99	0.98	0.89

To achieve this sample size, we would need to recruit approximately 6,000 suspected influenza cases and follow-up those who meet the specific criteria (in the majority of recruited subjects this would be a positive result on the QuickVue Influenza A+B rapid diagnostic test), and our reasoning is as follows. During the peak season we would conservatively anticipate that 50% of subjects with ILI symptoms, as we have defined these above, are infected with influenza rather than another virus (3,000 of the 6,000 tested). Recent international oseltamivir trials found that during the influenza peak season the proportion of subjects with influenza-like-illness who had confirmed influenza was 60% [29] and 66% [30]. Allowing for a sensitivity of 79% for the QuickVue test, only 2,370 index cases would be correctly identified (the remainder would be misclassified as false negatives). Given the estimated specificity of the QuickVue test of 92%, testing the 3,000 non-influenza index cases would result in misclassification of 240 subjects without influenza (false positives), and their households would also be visited. While the specificity of the symptom-based definition in 4(d) is likely to be lower, the sensitivity may be higher and with symptom-based recruitment only occurring in a small number of sites the approximate calculation above is appropriate. Thus we anticipate that we would need to recruit approximately 6,000 index cases with ILI symptoms and apply the specific criteria (typically the rapid diagnostic test), of who 1,477 (2,370+240) would meet our criteria and be subject to randomization and follow-up. Given the intention-to-treat approach, randomization and follow-up of some non-influenza cases is an expected consequence of the speed required by this study.

d) The level of significance to be used

We will use a significance level of α=0.05.

e) Criteria for the termination of the trial

Given the short duration of the trial, we do not plan to conduct any interim analyses or specify any early-stopping rules.

f) Procedure for accounting for any missing, unused and spurious data

The laboratory definition of influenza will be preferentially used as the primary outcome measure, but when this is unavailable we will use the clinical definition. In the pilot study, index cases and household contacts with missing data on important predictors will be excluded from analyses. In the main study, we will use multiple imputation [31] with 10 imputed datasets to replace missing values on outcome and predictor variables. If 10 imputed datasets are not sufficient to ensure stability of estimates we will use 20 imputed datasets. Multiple imputation makes maximum use of available data and maximises statistical power while requiring less strict theoretical assumptions than to a complete case analysis, or single imputation of mean values. We note that this is now one of the preferred (and standard) methods for analysing clinical trials data [32].

g) Procedures for reporting any deviations from the original statistical plan

Any deviations from the original statistical plan will be described and justified in the final report.

h) Selection of subjects to be included in the analyses

We will follow an intention-to-treat approach in the analyses. Households without a laboratory confirmed index case, and additionally in the main study those households where a household contact is laboratory confirmed to have influenza infection at baseline, will be excluded from the primary analysis.

10. Direct Access to Source Data

We will permit direct access to source data and documents for the purposes of trial-related monitoring, audits, IRB/IEC review and regulatory inspections. As required by the NIH/CDC funding agency, the *anonymised* individual participant data will be made publicly available after publication of our results in peer-reviewed journals and no later than 24 months after the conclusion of our study.

11. Ethics

An important ethical consideration is that households randomised to the control intervention might be considered to have less benefit from the trial than those assigned to the mask or hand washing interventions. However we note that there is little evidence that masks or hand washing can reduce influenza transmission, whereas this study will provide that evidence. Further, the participation of a control arm is essential to allow estimation of the effect of the interventions, given the lack of local-specific data on the SAR in typical circumstances. Thus we believe that those in the control arm, as the whole of society, will still benefit indirectly from this research.

12. Data Handling and Record Keeping

Socio-demographic and epidemiological data; confidential patient details (name address) will be collected from all subjects via the four questionnaires included in the appendix. All data will be anonymized when entered into an electronic database (double entry) and stored in the Department of Community Medicine, HKU. Original identities will be kept in a separate file accessible only to the trial manager. Original documents will be destroyed at the conclusion of the pilot study.

13. Financing and Insurance

This study is financed by a grant from the Centers for Disease Control and Prevention (Appendix A).

14. Publication Policy

The results will be published in international peer-reviewed journals.

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CONSORT Statement 2001 - Checklist

Items to include when reporting a randomized trial

PAPER SECTION And topic	Item	Descriptor	Reported on Page #
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned").	Abstract
INTRODUCTION Background	2	Scientific background and explanation of rationale.	Introduction
METHODS Participants	3	Eligibility criteria for participants and the settings and locations where the data were collected.	Methods
Interventions	4	Precise details of the interventions intended for each group and how and when they were actually administered.	Methods
Objectives	5	Specific objectives and hypotheses.	Methods
Outcomes	6	Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	Methods
Sample size	7	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules.	Methods
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification)	Methods
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	Methods
Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	Methods
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	Methods
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s); Methods for additional analyses, such as subgroup analyses and adjusted analyses.	Methods
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	Results
Recruitment	14	Dates defining the periods of recruitment and follow-up.	Results
Baseline data	15	Baseline demographic and clinical characteristics of each group.	Results
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat". State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	Results
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval).	Results
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	Results
Adverse events	19	All important adverse events or side effects in each intervention group.	Results
DISCUSSION Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	Discussion
Generalizability	21	Generalizability (external validity) of the trial findings.	Discussion
Overall evidence	22	General interpretation of the results in the context of current evidence.	Discussion