MAJOR ARTICLE

Effectiveness of Public Health Measures in Mitigating Pandemic Influenza Spread: A Prospective Sero-Epidemiological Cohort Study

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Background. Few studies have validated the effectiveness of public health interventions in reducing influenza spread in real-life settings. We aim to validate these measures used during the 2009 pandemic.

Methods. From 22 June to 9 October 2009, we performed a prospective observational cohort study using paired serum samples and symptom review among 3 groups of Singapore military personnel. "Normal" units were subjected to prevailing pandemic response policies. "Essential" units and health care workers had additional public health interventions (eg, enhanced surveillance with isolation, segregation, personal protective equipment). Samples were tested by hemagglutination inhibition; the principal outcome was seroconversion to 2009 influenza A(H1N1).

Results. In total, 1015 individuals in 14 units completed the study, with 29% overall seroconversion. Seroconversion among essential units (17%) and health care workers (11%) was significantly lower than that in normal units (44%) ($P < .001$). Symptomatic illness attributable to influenza was also lower in essential units (5%) and health care workers (2%) than in normal units (12%) ($P = .06$). Adjusted for confounders, unit type was the only significant variable influencing overall seroconversion ($P < .05$). From multivariate analysis within each unit, age ($P < .001$) and baseline antibody titer ($P = .012$) were inversely related to seroconversion risk.

Conclusions. Public health measures are effective in limiting influenza transmission in closed environments.

The 2009 influenza A(H1N1) pandemic affected most countries within months of its emergence. Despite early identification of the virus and massive scale-up of vaccine production, initial responses were largely based on preexisting pandemic plans due to the pandemic's rapid spread before the vaccine's availability months later. The use of combination strategies, including anti-viral treatment of cases, prophylaxis and quarantine of close contacts, and community social distancing have been shown in computational mathematical models to reduce influenza attack rates [1–3]. Although policy makers worldwide have adopted these interventions, there are few studies that have clearly validated these models' findings for pandemic influenza control in real-life settings, which is critical to assess their actual effectiveness [4].

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Influenza pandemic attack rates have been higher than those of nonpandemic influenza [5], whereas attack rates have also been high in environments such as military facilities (42%–58%) [6, 7] and boarding schools (71%) [8], even in nonpandemic years. There is indirect evidence that schools are potential amplifying arenas for influenza transmission [9, 10], and the same may also be true for other closed and semiclosed environments. Notably, the first outbreaks of 2009 influenza A(H1N1) in Singapore, a tropical city-state in Southeast Asia, occurred in such settings: a tertiary education institute, army camps, and church camps [11]. Therefore, measures that can control outbreaks early in closed or semiclosed environments may be an important pandemic mitigation strategy.

Most population-based studies of nonpharmacological interventions have been based on influenza-like illness (ILI) or laboratory-confirmed influenza [12, 13], but there are difficulties arising from lack of specificity of clinical definitions and documentation of laboratory-confirmed influenza. Sero-epidemiological studies are more definitive in determining population infection rates and effectiveness of interventions [14, 15], yielding critical information on preventive measures that cannot be obtained from observational studies alone. Previous pandemic studies have either relied on observational data [16, 17], single post-pandemic blood specimens [18], or cross-sectional serological samples [19]. However, the presence of cross-reactive antibodies to 2009 influenza A(H1N1) [19, 20] underscores the importance of using paired samples for precise measurement of seroconversion rates.

We therefore undertook a prospective observational study using paired serum samples among 3 distinct groups of military soldiers in Singapore to evaluate the impact of public health measures for the control of pandemic influenza before the pandemic vaccine was made available.

METHODS

This study was performed on the Singapore military from 22 June to 9 October 2009 and was part of a larger seroepidemiological investigation involving other cohorts—community adults, health care workers from 1 hospital, and long-term care facility residents [21]. Singapore is a globally connected tropical city-state, and the Singapore military is composed of both conscript personnel, where all men enlist after high school, and regular employees. These personnel work and reside in military camps during weekdays and return to the community on weekends, resulting in a mostly closed-living environment but with regular exposure to the general community.

The military's pandemic response plan was developed prior to the pandemic and involved a stratified and targeted response to ensure operational readiness. "Normal" units followed the prevailing national pandemic response policies, in which individuals were provided general health education on respiratory and hand hygiene and were advised to seek medical care if ill.

"Essential" units, defined as those units critical to the military's functioning where absenteeism at any time point must be minimized, received an additional set of public health measures during the epidemic's duration. This included enhanced surveillance (daily recorded temperature and symptom monitoring with prompt identification and reporting of acute respiratory illnesses [ARIs]) with medical referral and provision of home medical leave for these cases, as well as segregation of units into smaller working subgroups. Segregation as a form of social distancing entailed noncontact when possible between subgroups of as small as 20 individuals, including having different activity and meal times, and times of entry and exit from camp. Health care workers in military medical centers were subjected to similar enhanced surveillance measures as essential units and in addition wore N95 masks, gloves, and gowns continuously during their working hours; compliance was ensured through regular inspections. Both these groups also received routine annual seasonal influenza vaccination, which was not routinely offered to normal units, who were, however, free to obtain this on their own.

These 3 main groups were selected a priori to evaluate the effectiveness of the different levels of interventions summarized in Table 1. Multiple units representative of each main group were chosen, and participants were recruited from within these units. The working and living facilities, and intraunit interactions, were generally similar across the units.

Three blood samples were collected from each participant. The first sample (sample A) was taken from 22 June to 1 July 2009, immediately following the appearance of local community transmission in Singapore in the second half of June 2009 [22], before the widespread epidemic. None of the units selected had any recorded increase in respiratory illness or had any confirmed 2009 influenza A(H1N1) cases before sampling. The second sample (sample B) was mostly taken from 20 August to 3 September 2009, 3–4 weeks after the epidemic's peak in Singapore, which occurred during the first week of August 2009 [23, 24]. The final postepidemic sample (sample C) was taken from 29 September to 9 October 2009, 4–5 weeks after epidemic activity had subsided and national ARI and ILI rates had returned to baseline levels [23, 24].

Standardized questionnaires were given to participants during the 3 blood samplings, and at 3-week intervals in between. The questionnaires collected data on demographics, medical history, any previous vaccination history, and new onset symptoms related to influenza. ARI was defined as onset of rhinorrhea, nasal congestion, sore throat or cough; febrile respiratory illness (FRI) was defined as ARI with concurrent self-reported fever or temperature of $\geqslant$37.5°C [25]. Written informed consent was obtained, and study approval was granted by the mil-

Table 1. Characteristics of Study Population

Characteristic	Overall	Normal personnel	Essential personnel	Health care workers
No. of units in each cohort	14	5	5	4
Total no. of individuals in the units	1515	594	757	164
No. of individuals who participated	1166 (77)	472 (79)	567 (75)	127 (77)
Mean sample size who participated per unit (SE)	73 (11)	87 (4)	94 (21)	27 (7)
Samples				
At least 2 samples provided	1015/1166 (87)	437/472 (93)	470/567 (83)	108/127 (85)
Interventions		Standard pandemic plan	Standard pandemic plan plus	Standard pandemic plan plus
			• Segregation among working subgroups	• PPE, including N-95 during working hours
			• Enhanced surveillance and isolation[a]	• Enhanced surveillance and isolation [a]
			• Seasonal influenza vaccination	• Seasonal influenza vaccination
Demographics				
Median age[b]	20	19	22	21
IQR	19–22	18–20	20–26	20–22
Range	17–61	17–51	18–61	18–54

NOTE. Data are no. (%) of participants, unless otherwise indicated. IQR, interquartile range; PPE, personal protective equipment; SE, standard error.

[a] Included daily temperature monitoring and prompt reporting of cases of acute respiratory illness, with provision of home medical leave.

[b] Significant at the $P < .001$ level.

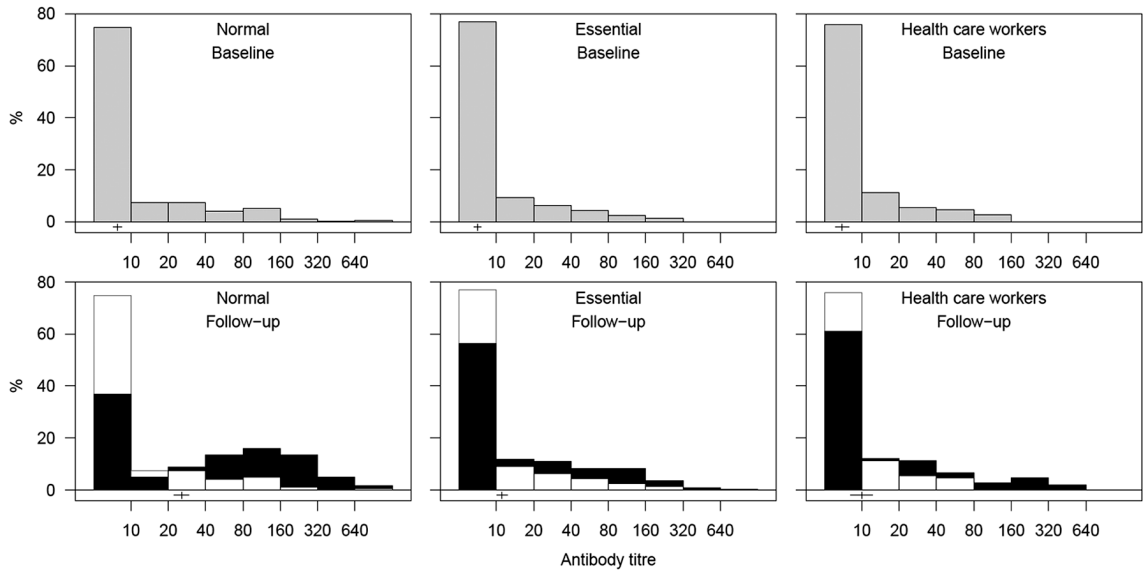

Figure 1. Proportion of participants with the respective baseline and follow-up antibody titers among the 3 groups. For the follow-up graphs, the black bars are the follow-up antibody titers, whereas the white bars are the baseline titers superimposed for comparison. Geometric mean titers are shown in the row below the bars, with the mean value indicated by the vertical line and 95% confidence interval indicated by the horizontal line.

itary's Joint Medical Committee for Research and the Australian National University's ethics review board.

Laboratory methods. For each sampling, 5–10 mL of venous blood was taken. The hemagglutination inhibition assay was performed for all samples in parallel, according to standard protocols [26] at the World Health Organization (WHO) Collaborating Centre for Reference and Research for Influenza in Melbourne, Australia.

The serum was pretreated with receptor destroying enzyme (RDE [II]; Deka Seiken Co Ltd), 1:4 (vol/vol), at 37°C for 16 h; then the enzyme was inactivated by addition of an equal volume of 1.5% tri-sodium citrate (Ajax Chemicals) and incubation at 56°C for 30 min. Egg-grown A/California/7/2009 A(H1N1–2009) virus was purified by sucrose gradient, concentrated, and inactivated with β-propiolactone, to create an influenza zonal pool (IZP) preparation. In total, 25 μL of (4HAU) IZP-A/California/7/2009 virus was incubated at room temperature with an equal volume of RDE-treated serum. Serum samples were titrated in 2-fold dilutions in phosphate-buffered saline from 1:10 to 1:1280. Following 1 h incubation, 25 μL of 1% (vol/vol) turkey red blood cells was added to each well. Hemagglutination inhibition was read after 30 minutes. Titers were expressed as the reciprocal of the highest dilution of serum where hemagglutination was prevented.

To determine seroconversion to 2009 influenza A(H1N1) in individual participants, we compared antibody titers between successive pairs of blood specimens. Seroconversion was defined as a $\geqslant$4-fold rise in antibody titers [27].

Statistical analysis. The principal outcome measure was seroconversion to 2009 influenza A(H1N1). The secondary outcome measure was the presence of ARI and/or FRI likely due to influenza (ie, symptomatic seroconversions). To determine the sample size, we assumed 25% seroconversion in normal units on the basis of previous studies and a difference with the intervention groups of at least 10%. To achieve 80% statistical power at the 5% significance level for pairwise comparisons

Table 2. Rates of Seroconversion and Symptomatic Seroconversion among the 3 Groups

Unit type	Seroconversion			Symptomatic seroconversion, mean proportion (SE)[b]
	Mean proportion (SE)	Relative risk (95% CI) [a]	*P* value[a]	
Normal	44 (3)	1	N/A	12 (3)
Essential	17 (3)	0.39 (0.26–0.54)	<.001	5 (3)
Health care worker	11 (3)	0.26 (0.07–0.46)	<.001	2 (3)

NOTE. CI, confidence interval; N/A, not applicable; PPE, personal protective equipment; SE, standard error.

[a] Comparing the intervention cohorts (essential cohort or health care cohort) to the nonintervention normal cohort.

[b] $P = .061$, comparing all 3 groups using the Kruskal-Wallis test.

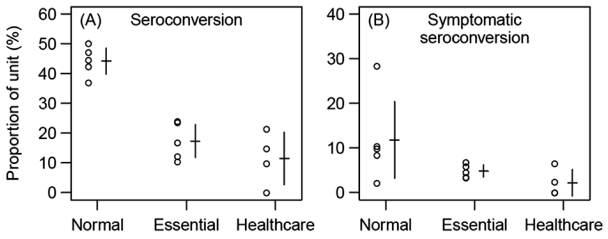

Figure 2. Rates of seroconversion (*A*) and symptomatic seroconversion (*B*) within individual units among the 3 groups.

would require 250 independent participants per group. At the unit level, a postulated mean difference of 10% and 5% standard deviation require 5 units to attain the same power and significance level. We therefore aimed for 250 individuals in each comparison group, spread among 5 units per group. With dependence of final outcomes, sample sizes would have to increase; however, logistical constraints resulted in recruitment of more normal and essential soldiers but fewer health care workers.

Demographics were compared using the Kruskal-Wallis test for age and Pearson χ^2 test for sex, using the Hope Monte Carlo version for small sample sizes with 1 million iterations [28]. The Pearson χ^2 test was also used to assess whether baseline titers varied by seasonal vaccination status. We also computed the geometric mean titers (GMTs) for the 3 groups, where titers <10 were assigned a value of 5.

Other statistical analyses were based on the dependent nature of disease status within units due to the contagious nature of influenza. For the main analysis, we therefore treated the military unit as the basic statistical unit of interest, implicitly assuming that individual military units are effectively independent—their members working and living separately from other military units. Overall unit-level seroconversion rates in the 3 groups were compared using a Fisher analysis of variance (ANOVA) test with Gaussianness tested by the Shapiro-Wilk test [29] and homoskedasticity by the Bartlett test [30]. Confidence intervals (CIs) for relative risks (RRs) were calculated using the Fieller method [31].

Finally, we performed 2 multivariate analyses. The first was an ecological study on unit seroconversion using properties of the unit as predictors, namely, mean age, proportion of men, and antibody titer at baseline, within a linear model framework to assess whether these confounded unit type. In addition, an individual-level multiple logistic regression was performed using age, sex, titer at baseline, and vaccine status (the only intervention component that could be separately quantified) as predictors along with a unit-specific dummy variable, allowing the possible effect of these covariates to be assessed, conditioned on attack rates within each unit. All analyses were performed with R software programming language [32].

RESULTS

Study population. A total of 1515 individuals from 14 different military units were initially selected. Of these, 1166 (77%) agreed to participate in the study, and 87% (1015/1166) of participants completed the study by providing a baseline and at least 1 other sample for paired sample serological analysis. The majority who did not complete the study were soldiers who had left the military during the study. The follow-up rates for the normal, essential, and health care worker cohorts were 93% (437/472), 83% (470/567), and 85% (108/127), respectively.

Table 1 shows the general characteristics of the cohort. The majority of the participants were young men, reflecting the general military population. Essential units had a significantly higher proportion of personnel of older ages compared with the other groups ($P < .001$). Health care worker units were smaller than the other units because these were teams working in primary health care facilities that had fewer personnel than other training or working units.

Primary and secondary outcomes. The baseline and follow-up antibody titers among the 3 groups are shown in Figure 1. There was no significant difference in the baseline titers among the 3 groups, whereas the GMT on follow-up were significantly higher in normal personnel than in the other cohorts ($P < .001$). The mean fold increase in titers comparing baseline to follow-up titers for each individual was 1.65 (95% CI, 1.53–1.77) for essential personnel, 1.52 (95% CI, 1.30–1.73) for health care workers, and 2.72 (95% CI, 2.52–2.91) for normal personnel ($P < .001$).

Overall, the primary outcome of serologically confirmed 2009 influenza A(H1N1) infection was 29% (295/1015). Mean serologically confirmed infections were significantly lower in both essential worker units (17%; $P < .001$) and health care worker units (11%, $P < .001$) than in the normal units (44%) (Table 2). However, no significant difference was found between essential units and health care worker units (RR, 0.66; 95% CI, 0.14–1.54; $P = .22$). The secondary end point of symptomatic illness attributable to influenza infection was lower among essential units (4.8%) and health care workers (2.2%) than in normal units (12%) and of borderline statistical significance ($P = .061$). Figure 2 shows the rates of seroconversion and symptomatic seroconversion within individual units among the 3 cohorts, whereas the exact breakdown by individual units can be found in Table 3.

Performing a multivariate, ecological, unit-level analysis with unit type, proportion of men, mean age, and baseline antibody

Table 3. Rates of Seroconversion and Symptomatic Seroconversion in Each Individual Unit

This table is available in its entirety in the online version of *Journal of Infectious Diseases*.

Table 4. Multivariate Logistic Regression of Factors Affecting Seroconversion within Each Individual Unit

Predictor	OR	95% CI	*P* value
Age, years	0.86	(0.80–0.94)	<.001
Sex, men relative to women	2.73	(0.56–13.23)	0.21
Seasonal influenza vaccine	1.03	(0.63–1.68)	0.89
Baseline titer	0.80	(0.68–0.95)	.012

NOTE. CI, confidence interval; OR, odds ratio.

titers against 2009 influenza A(H1N1) as predictors of proportion who seroconverted, we found that only unit type was a significant predictor, with both essential units and health care units having lower proportions infected than did normal units (essential vs normal, $P = .007$; health care vs normal, $P = .001$). Age ($P = .59$), sex ($P = .89$), and baseline titer ($P = .74$) had no effect at this scale. This suggests that although age and sex patterns in the units differ, these differences did not result in different seroconversion rates other than those attributable to the associated interventions themselves.

To assess whether age, sex, or baseline antibody titers had any protective effect at the individual level, we performed a multiple logistic regression with a separate intercept parameter per unit. Seasonal vaccine status, the only intervention we could separately quantify, was also included as a parameter. Table 4 summarizes this multivariate analysis and the corresponding odds ratios. Age ($P < .001$) and initial titer ($P = .012$) were significantly inversely related to the risk of seroconversion after accounting for different exposures in the different units, although there was no evidence of effect on the basis of sex ($P = .21$). Seasonal vaccine at the individual level was also not a significant predictor of individual seroconversion ($P = .89$). In addition, there was no significant difference in baseline geometric mean antibody titers (GMTs) between those who received previous seasonal vaccine and those without (11.2 vs 15.1; $P = .15$).

Epidemic curves. Figure 3 shows the cumulative clinical epidemic curves based on reported symptom onset of ARI and/or FRI among those who seroconverted, stratified by unit type and compared to the estimated community cumulative epidemic curve [24]. The peak of the epidemic for normal units occurred earlier than in the essential units, health care worker units, and the general community.

CONCLUSIONS

This is one of the first large serological cohort studies to document the effectiveness of combined public health interventions against pandemic influenza. These interventions can be performed with minimal disruption of essential services and potentially reduce the impact of influenza illness and transmission, leading to lower peak infection rates and point absenteeism. Delaying disease spread may allow other interventions to be instituted, such as vaccination that was only available later. Our normal units had earlier epidemic peak than did the general community and high overall seroconversion rates (44%), which are likely due to the younger adult ages and closed settings. A similar observation was made in seasonal influenza outbreaks in schools (21%–71%) [8, 33] and military camps (42%–58%) [6, 7], and during the 2009 pandemic with >30% attack rate during a school outbreak [34]. It is therefore important to reduce influenza transmission in similar closed settings where high attack rates during a short time period and high absenteeism are undesirable, including schools, boarding facilities, long-term elderly care facilities, and essential services such as health care workers. This may also reduce the chance of such settings acting as amplifiers for a novel virus [9, 10, 35].

Our study showed the likely effectiveness of public health measures, in particular, enhanced surveillance (daily temperature taking and prompt ARI reporting) with isolation through home medical leave, and segregation of smaller subgroups, on the spread of influenza. These measures were effective, easy to administer, and sustainable during the entire 2-month epidemic. Symptomatic seroconversion was also reduced in the intervention cohorts with marginal significance, suggesting that the interventions proportionally reduced symptomatic cases (a proxy for absenteeism). Although seasonal influenza vaccination may elicit cross-reactive antibodies against the pandemic strain [20], there was no evidence in our study that individuals vaccinated against seasonal influenza had lower risk of infection above the other interventions. Essential units had delayed onset compared

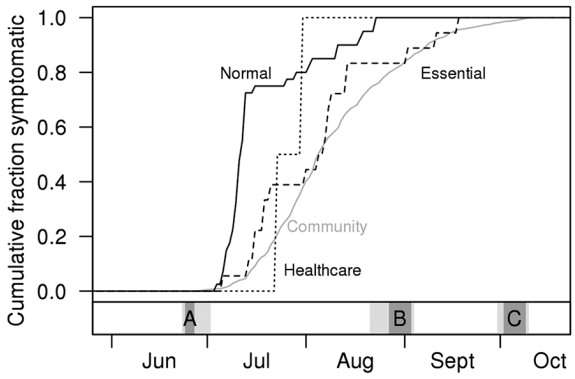

Figure 3. Cumulative clinical epidemic curves among those who seroconverted for each group, compared with estimated community cumulative epidemic curve from GP sentinels. Colored bands indicate sampling times, with dark bands representing interquartile range (IQR) and light bands representing range (excluding 11 samples taken from 9 to 10 September 2009). Multiple acute respiratory illness and/or febrile respiratory illness episodes per individual were proportioned equally and attributed to that individual.

with normal units but similar to that of the community (Figure 3), possibly due to community exposures and the effectiveness of the public health measures in delaying spread in the military setting.

Our results provide serological evidence to support previous observational studies during pandemics where public health measures designed to reduce transmission such as social distancing and restrictions on public gatherings, and isolation and quarantine significantly reduced overall mortality in the absence of an efficacious vaccine [16, 17, 36]. The measures used in our study were minimally disruptive and ensured business continuity by minimizing peak infection rates and point absenteeism and can be similarly applied to other closed settings over long durations with prior planning. For example, in schools, daily temperature taking and symptom monitoring can be implemented on entry, and anyone with respiratory illnesses can be referred for medical consult. In addition, social distancing can be achieved through students being segregated by classrooms or educational levels with staggered entry and exit, breaks and meal times, and deferment of school-wide mass gatherings. These measures can potentially reduce simultaneous spread across classes, without the need for disruptive school closures.

Health care workers had reduced attack rates compared with normal units possibly due to the use of personal protective equipment, including wearing of N-95 masks during working hours, on top of the enhanced surveillance. Health care workers also had lower seroconversion rates compared with essential units, although this was not statistically significant (RR, 0.66; $P = .22$). At the same time, 11% of health care workers showed serological evidence of infection, highlighting the possible role played by nonoccupational acquisition of influenza. Although health care staff would have similar infection risks from settings outside their work environment, they might reasonably be expected to have higher occupational exposure to pandemic influenza cases and thus would have been expected to have had higher infection risks. The combination of personal protective equipment, together with enhanced surveillance, may have reduced seroconversion rates among health care workers despite their higher risk exposure. These strategies may be similarly applied to health care workers in other settings, reducing their risk of infection and minimizing disruption to the critical provision of health care.

We have also found that age and baseline antibody titers were independently inversely correlated with seroconversion. This is consistent with other observations that the pandemic affects young adults with relative sparing of older age groups [19, 35]. A recent study found that preexisting antibodies may protect against pandemic influenza infection [19], whereas higher baseline titers may also independently reduce the likelihood of infection and consequently seroconversion [20, 37, 38]. Additional studies are needed to determine the effectiveness of baseline antibodies in reducing influenza infection.

Limitations of this study include the relatively few groups for comparison and the study's inability to separate the incremental impact of each individual intervention. These are intrinsic issues with observational cohort studies, even preplanned ones like ours, and additional studies should aim to look at other interventions, whether in combination or individually, where opportunities exist. We did not monitor participants after the epidemic to determine if cumulative case numbers trended toward parity over time for the different groups. However, national and military surveillance data showed that postepidemic ILI rates and percentage of ILI positivity for 2009 influenza A(H1N1) were low, and no major pandemic influenza outbreaks were detected in the military.

The measures adopted by the Singapore military were simple to implement rapidly, and the data reported here suggest that these public health measures—in particular, enhanced surveillance with isolation and social segregation—are likely to be effective in limiting influenza transmission and reducing the high attack rates during an epidemic in closed environments. These should be considered in preparation for future epidemics and pandemics, as well as in developing countries where pandemic vaccine coverage has not reached sufficient levels to prevent future outbreaks.

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