ORIGINAL CONTRIBUTION

2009 Influenza A(H1N1) Seroconversion Rates and Risk Factors Among Distinct Adult Cohorts in Singapore

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Context Singapore experienced a single epidemic wave of 2009 influenza A(H1N1) with epidemic activity starting in late June 2009 and peaking in early August before subsiding within a month.

Objective To compare the risk and factors associated with H1N1 seroconversion in different adult cohorts.

Design, Setting, and Participants A study with serial serological samples from 4 distinct cohorts: general population (n=838), military personnel (n=1213), staff from an acute care hospital (n=558), and staff as well as residents from long-term care facilities (n=300) from June 22, 2009, to October 15, 2009. Hemagglutination inhibition results of serum samples taken before, during, and after the epidemic and data from symptom questionnaires are presented.

Main Outcome Measures A 4-fold or greater increase in titer between any of the 3 serological samples was defined as evidence of H1N1 seroconversion.

Results Baseline titers of 40 or more were observed in 22 members (2.6%; 95% confidence interval [CI], 1.7%-3.9%) of the community, 114 military personnel (9.4%; 95% CI, 7.9%-11.2%), 37 hospital staff (6.6%; 95% CI, 4.8%-9.0%), and 20 participants from long-term care facilities (6.7%; 95% CI, 4.4%-10.1%). In participants with 1 or more follow-up serum samples, 312 military personnel (29.4%; 95% CI, 26.8%-32.2%) seroconverted compared with 98 community members (13.5%; 95% CI, 11.2%-16.2%), 35 hospital staff (6.5%; 95% CI, 4.7%-8.9%), and only 3 long-term care participants (1.2%; 95% CI, 0.4%-3.5%). Increased frequency of seroconversion was observed for community participants from households in which 1 other member seroconverted (adjusted odds ratio [OR], 3.32; 95% CI, 1.50-7.33), whereas older age was associated with reduced odds of seroconversion (adjusted OR, 0.77 per 10 years; 95% CI, 0.64-0.93). Higher baseline titers were associated with decreased frequency of seroconversion in community (adjusted OR for every doubling of baseline titer, 0.48; 95% CI, 0.27-0.85), military (adjusted OR, 0.71; 95% CI, 0.61-0.81), and hospital staff cohorts (adjusted OR, 0.50; 95% CI, 0.26-0.93).

Conclusion Following the June-September 2009 wave of 2009 influenza A(H1N1), 13% of the community participants seroconverted, and most of the adult population likely remained susceptible.

JAMA. 2010;303(14):1383-1391 www.jama.com

On April 24, 2009, the World Health Organization (WHO) reported the emergence of a novel influenza A virus (2009 influenza A[H1N1]).[1] Early data from Mexico based on laboratory-confirmed cases suggested higher infection rates in younger age groups but higher case-fatality ratios in elderly individuals,[2] although it was initially unclear whether these observations were affected by biases in case ascertainment. Various experts have called for serological investigations to more accurately determine infection rates, especially since a substantial proportion of influenza infections are asymptomatic.[3]

Singapore, a Southeast Asian tropical city-state of 4.8 million people and a global travel hub, detected its first imported cases of 2009 influenza A(H1N1) in late May 2009. Virological surveillance documented sustained community transmission from the latter half of June 2009,[4-7] followed by a single epi-

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demic wave peaking in the first week of August and subsiding by September 2009.[7,8] We initiated a cohort study using serial blood specimens to determine antibody levels against 2009 influenza A(H1N1) as a marker of infection in 3 different population groups of public health concern—military personnel, acute care hospital workers, and staff members and residents of long-term care facilities and compared them with community-dwelling adults. The study aimed to compare the risk of infection in these different cohorts and to investigate risk factors for infection.

METHODS

Study Design

This was a cohort study including 4 different populations in Singapore and involving the planned collection of up to 3 serial serological samples from each individual: a baseline sample was collected either before the local 2009 influenza A(H1N1) epidemic using banked samples or in the early epidemic phase before widespread community transmission; the second sample was collected during the epidemic about 4 weeks after the epidemic had peaked; and third sample was collected at least 4 weeks after epidemic activity had subsided.

When possible, the start and stop dates of specimen collection across the cohorts were intentionally synchronized to allow intercohort comparison of seroconversion rates at each follow-up time point. Clinical symptom reviews were performed using a standardized questionnaire once every 2 weeks for the community cohort and at each sample collection in the other 3 cohorts. Participants were asked to report all new-onset respiratory symptoms and constitutional symptoms such as headaches, myalgia, and fever (including measured temperature where available); and baseline demographic data and whether they had ever received seasonal influenza vaccination in the past.

Study Populations

1. Community-dwelling adults were recruited from the Multiethnic Cohort (MEC) of the Singapore Consortium of Cohort Studies (SCCS), a long-term research project initiated to study gene-environment interactions in chronic disease causation. The MEC (http://www.nus-cme.org.sg/home.html) is a subcohort of the SCCS, comprising about 9000 community-dwelling healthy Singaporeans aged 21 to 75 years, recruited through public outreach activities and referrals for which recruitment is ongoing. We enrolled new MEC recruits into the study (from late June 2009), and recontacted 2400 existing MEC participants, with the aim of enrolling 900 participants. For the first serum sample collection, new recruits donated fresh baseline blood, while existing participants granted permission to use specimens banked on original recruitment. Symptom questionnaires were administered via telephone interviews at 2-week intervals.

2. The military personnel cohort was recruited from the Singapore Armed Forces, Singapore's national military and composed largely of conscripted males who serve after completion of high school from ages 18 through 19 years. Most individuals reside in military camps during weekdays but return to the community on weekends. Individuals were recruited by invitation from 15 units selected to give a good representation of the entire military structure, with a total personnel of 1570. Blood samples were taken at all 3 time points together with self-administered questionnaires.

3. Hospital staff from Tan Tock Seng Hospital, an acute care hospital with 6000 staff members, formed the third cohort. Staff members were recruited through e-mail notifications and by word-of-mouth referrals. Blood samples were taken at all 3 time points along with self-administered questionnaires. Information on symptomatic episodes was augmented through sickness absenteeism records for details such as dates of illness.

4. Staff and residents from 2 long-term care facilities, Jamiyah Home for the Aged and Peacehaven Nursing Home, were recruited by invitation. Between the 2 facilities are a total of 179 staff members and 520 residents (200 residents were able to give consent) who rarely go outside the facility. In this cohort, only the first and third serum samples were taken, with questionnaires simultaneously administered by trained interviewers.

Specimen Collection and Laboratory Methods

Venous blood was taken in 5- to 10-mL plain tubes. Serum samples were pretreated with receptor destroying enzyme (RDE [II], Deka Seiken Co Ltd, Tokyo, Japan), 1:4 (vol/vol), at 37°C for 16 hours, before enzyme inactivation by the addition of an equal volume of 1.6% trisodium citrate (Ajax Chemicals, Melbourne, Australia) and incubation at 56°C for 30 minutes.

The hemagglutination inhibition assay was performed according to standard protocols at the World Health Organization Collaborating Centre for Reference and Research on Influenza in Melbourne, Australia.[9] Egg-grown A/California/7/2009 A(H1N1) pandemic virus was purified by sucrose gradient, concentrated and inactivated with β-propiolactone, to create an influenza zonal pool preparation (a gift from CSL Limited, Melbourne, Australia). Twenty-five microliters (4 hemagglutination units) of influenza zonal pool-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 hour of 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. We defined seroconversion as a 4-fold or greater increase in antibody titers.

The hemagglutination inhibition assay was assessed on paired serum samples from 56 cases of 2009 influenza A(H1N1) that were confirmed through reverse transcription–polymerase chain reaction (RT-PCR): 28 participants from this

cohort study, plus 7 outbreak-related cases and 21 clinical cases admitted to Tan Tock Seng Hospital. Forty-five patients (80%) seroconverted to the pandemic strain, A/California/7/2009. Only 20 patients (20%) seroconverted to A/Brisbane/59/2007(H1N1) and 7 (13%), to A/Wisconsin/15/2009(H3N2).

Data Analysis and Statistics

Participants who seroconverted between any successive pairs of blood specimens (either from baseline to the second sample, second to third sample, or first to third sample) were considered as ever having had serological evidence of infection during the study period. Geometric mean titers (GMTs) were estimated by assigning a value of 5 for titers lower than 10 and a value of 1280 for titers of 1280 or higher.

Episodes of acute respiratory illness were defined as new-onset illness with any respiratory symptoms of rhinorrhea, nasal congestion, sore throat, or cough; and febrile respiratory illness was defined as an acute respiratory episode with self-reported fever or a body temperature of 37.5°C or higher. The date of each illness episode was the earliest symptom onset date or sickness absenteeism if onset dates were unavailable. Febrile respiratory illness episodes that preceded seroconversion were graphed by illness date against influenza epidemic activity. Likewise, illness episodes preceding seroconversion were used to estimate the proportion of seroconverting individuals with acute respiratory or febrile respiratory illness episodes. Singapore influenza epidemic activity data were from laboratory surveillance on the weekly proportion of influenza-like illness general practice samples testing positive for 2009 influenza A(H1N1) and the weekly number of influenza-like illness consults seen by a separate sentinel general practice network[7,8]—the 2 data sets were multiplied to give a weekly epidemic curve.

As some participants formed natural groupings, such as households and military units, and as contagious disease status is nonindependent within groups, we accounted for nonindependence using dummy variables corresponding to disease status of others within the group. For the community cohort, we introduced 2 indicator variables coding for 3 categories corresponding to known seroconversion status for other individuals in the household—at least 1 other household member seroconverted, no one else in the household seroconverted, or other permutations (no other household member in the study or other members in the study but seroconversion not known). For military camps, a variable indicating the proportion of the other unit members who seroconverted was introduced. The same was done for hospital staff, using functional operating units (75 wards or departments). We then performed

Table 1. Cohort Characteristics

	Community	Military	Hospital Staff	Long-term Care
Timing of blood draws in 2009				
Baseline	June 22-27[a]	June 22-July 1	June 22-July 7	July 17-27
Second	August 20-29	August 20-September 3[b]	August 19-September 3	NA
Third	October 6-11	September 29-October 9	September 29-October 15	October 5-7
Samples, No. (%) of participants				
Baseline	838 (100)	1213 (100)	558 (100)	300 (100)
Second	621 (74)	920 (76)	501 (90)	NA
Third	689 (82)	776 (64)	467 (84)	250 (83)
All 3 samples	583 (70)	636 (52)	431 (77)	NA
≥2	727 (87)	1060 (87)	537 (96)	250 (83)
No. (%) of reviews completed[c]	4766 (95)	1680 (69)	1098 (98)	250 (83)
Age, mean (range), y	43 (21-74)	22 (17-62)	34 (20-67)	56 (18-109)
Age in years, No. (%)				
15-19	0	554 (46)	0	12 (4)
20-24	92 (11)	473 (39)	110 (20)	28 (9)
25-29	66 (8)	93 (8)	129 (23)	24 (8)
30-39	152 (18)	44 (4)	164 (29)	47 (16)
40-49	298 (36)	31 (3)	96 (17)	14 (5)
50-59	166 (20)	14 (1)	52 (9)	32 (11)
≥60	64 (8)	4 (<1)	7 (1)	143 (48)
Sex, No. (%)				
Male	353 (42)	1175 (97)	92 (16)	131 (44)
Female	485 (58)	38 (3)	466 (84)	169 (56)
Seasonal influenza vaccine, No. (%)				
No	729 (87)	696 (57)	52 (9)	160 (53)
Yes	109 (13)	517 (43)	506 (91)	140 (47)

Abbreviation: NA, not applicable.
[a] Specimen collection dates for 23 community cohort participants; baseline samples for the remaining 815 participants used specimens banked on original recruitment into ongoing research study on chronic disease causation.
[b] Excludes 11 samples taken on September 9 and 10, 2009.
[c] Denominator is based on baseline multiplied by number of scheduled follow-up reviews: 6 for community, 2 for military, 2 for hospital staff, 1 for long-term care.

univariate and multivariate logistic regression using these dummy variables alongside baseline titer, age, sex, and seasonal influenza vaccine status to assess their contribution to seroconversion; odds ratios (ORs) with asymptotic Wald 95% confidence intervals (CIs) and 2-sided *P* values are presented with statistical significance set at the .05 level.[10] Multivariate analysis involved stepwise logistic regression, wherein variables that did not improve model fit at $P < .10$ were discarded.

A sample size of 450 participants per cohort was needed to give a power of 90% to detect (with a 2-sided *P* value of <.05) seroconversion rates that were 10% higher for a given cohort than the community sample, which was assumed would have seroconversion rates of 20% to 30% (similar to the 1957 pandemic[11]). Target sample sizes were 600 for hospital staff and long-term care facility cohorts and 900 for the community cohort to allow for loss to follow-up rates of 25% and 50%, respectively. The military cohort was substantially larger to allow comparison of seroconversion rates in different military units.

Where appropriate, 95% CIs for proportions were computed using the Wilson score-based method.[12,13] All statistical analyses were performed using STATA 10.0 (StataCorp, College Park, Texas).

Ethics Review

Written informed consent was obtained from all participants. The study was approved by the ethics review boards of the National Healthcare Group, Singapore Armed Forces, and National University of Singapore.

RESULTS

TABLE 1 describes the 4 cohorts. We completed baseline collection from 838 community participants by June 27, 2009, 1213 military participants by July 1, 2009, and 558 hospital participants by July 7, 2009, after simultaneously starting recruitment on June 22, 2009. The community cohort–banked samples dated back to June 2005, with 790 of 838 specimens (94%) collected before May 26, 2009, when the first imported influenza 2009 A(H1N1) case was detected in Singapore.[4] Logistical difficulties delayed baseline collection of the 300 long-term care facilities cohort participants until July 27, 2009, but there were no confirmed cases or excess influenza-like illness in either long-term care facility before the collection date. All participants (except those from the long-term care facilities cohort) were recalled for the second sample collection between August 19 and September 3, 2009, and the third sample collection between September 29 and October 15, 2009. In each cohort, 80%

Table 2. Baseline Titers by Cohorts, by Seasonal Influenza Vaccination in All Cohorts, and by Age Groups in the Community Cohort

	No. of Participants	Distribution of Antibody Titers, No. (%)			GMT (95% CI)	*P* Value
		<10[a]	10-20	≥40		
Cohort						
Community	838	738 (88)	78 (9)	22 (3)	5.8 (5.6-6.0)	
Military	1213	921 (76)	178 (15)	114 (9)	7.4 (7.1-7.7)	<.001[b]
Hospital staff	558	351 (63)	170 (30)	37 (7)	7.6 (7.2-8.1)	<.001[b]
Long-term care facilities	300	252 (84)	28 (9)	20 (7)	6.4 (6.0-6.9)	.007[b]
Seasonal influenza vaccine						
Community						
No	756	666 (88)	73 (10)	17 (2)	5.8 (5.6-6.0)	.34[c]
Yes	82	72 (88)	5 (6)	5 (6)	6.1 (5.3-7.1)	
Military personnel						
No	696	538 (77)	91 (13)	67 (10)	7.4 (6.9-7.9)	.98[c]
Yes	517	383 (74)	87 (17)	47 (9)	7.4 (6.9-7.9)	
Hospital staff						
No	52	39 (75)	11 (21)	2 (4)	6.4 (5.6-7.4)	.07[c]
Yes	506	312 (62)	159 (31)	35 (7)	7.8 (7.3-8.3)	
Long-term care facilities						
No	160	141 (88)	9 (6)	10 (6)	6.1 (5.5-6.7)	.14[c]
Yes	140	111 (79)	19 (14)	10 (7)	6.8 (6.1-7.7)	
Age groups in community cohort, y						
20-24	92	73 (79)	14 (15)	5 (5)	6.7 (5.8-7.7)	.002[d]
25-29	66	53 (80)	10 (15)	3 (5)	6.6 (5.6-7.7)	
30-39	152	136 (89)	14 (9)	2 (1)	5.6 (5.3-6.0)	
40-49	298	270 (91)	20 (7)	8 (3)	5.7 (5.4-6.0)	
50-59	166	148 (89)	15 (9)	3 (2)	5.7 (5.3-6.1)	
≥60	64	58 (91)	5 (8)	1 (2)	5.5 (5.0-6.1)	

Abbreviations: CI, confidence interval, GMT, geometric mean antibody titers.
[a] No detectable antibodies.
[b] Compared with community cohort using unpaired *t* test.
[c] Participants who did not have seasonal influenza vaccine compared with those who did using unpaired *t* test.
[d] Using linear regression with age as an explanatory value for GMT.

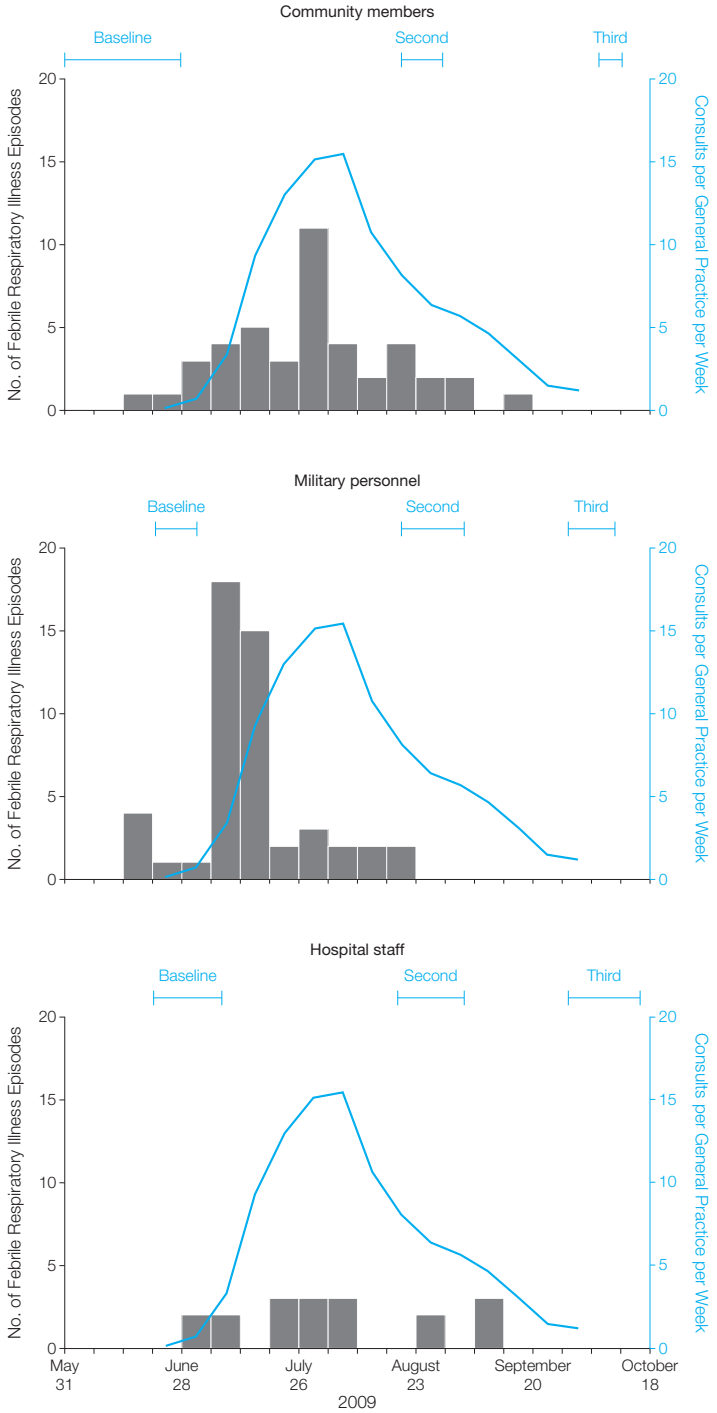
or more returned for at least 1 follow-up sample so that, except for the long-term care facilities cohort, the final number of participants for which seroconversion data was available exceeded our targeted sample sizes. Scheduled follow-up symptom reviews were also reasonably complete except in the military for which follow-up reviews were restricted to those with follow-up blood samples.

Military personnel were a mean age of 22 years (range, 17-62 years); hospital staff, 34 years (range, 20-67 years); and the community cohort, 43 years (range, 21-74 years), whereas the long-term care cohort, of which 54% (162/300) were residents, were a mean age of 56 years (range, 18-109 years). Sex distributions reflect the predominantly male workforce in the military (97%, 1175/1213) and female workforce in the hospital staff (84%, 466/558). Only 13% (109/838) in the community cohort had previously ever received seasonal influenza vaccine compared with 91% (506/558) of hospital staff, 43% (517/1213) of military, and 47% (140/300) of participants from long-term care facilities.

The baseline GMT for hospital staff was 7.6 (95% CI, 7.2-8.1); military personnel, 7.4 (95% CI, 7.1-7.7); and staff and residents of long-term care facilities, 6.4 (95% CI, 6.0-6.9), all of which were significantly higher than those of the community cohort: 5.8 (95% CI, 5.6-6.0; TABLE 2). The GMT of in-hospital staff who had received a seasonal influenza vaccine was 7.8 (95% CI, 7.3-8.3); whereas the GMT of staff who had not received the seasonal vaccine was 6.4 (95% CI, 5.6-7.4; $P=.07$). In the largely unvaccinated community cohort, younger age was significantly associated with higher baseline titers ($P=.002$).

The FIGURE shows that the epidemic curve peak for the community cohort coincided with the national peak in influenza epidemic activity, whereas the military personnel epidemic peaked 2 to 3 weeks earlier. Seroconversion occurred mostly between the baseline and the second sample for the community

Figure. Epidemic Curves for Each Cohort Constructed From Febrile Respiratory Illness Episodes in Seroconverting Participants



Braces represent the sampling periods and are compared against influenza epidemic activity as observed through H1N1-2009 general practice sentinel data. The H1N1-2009 general practice sentinel surveillance data are constructed by multiplying the proportion of laboratory surveillance isolates that tested positive for H1N1-2009 from the Ministry of Health and the number of influenza-like illness consults per general practice from a sentinel general practice network,[7,8] which gives the estimated number of general practice influenza-like illness consults that are influenza 2009 A(H1N1) for that week. Epidemic activity appears to have peaked in the week starting on August 2, 2009, at an estimated 15.5 consults per general practice per week.

with 70 of 98 eventual seroconversions (71%; 95% CI, 62%-79%) and for the military cohorts with 254 of 312 seroconversions (81%; 95% CI, 77%-85%) compared with hospital staff with 16 of 35 seroconversions (46%; 95% CI, 30%-62%) (TABLE 3). In the long-term care facilities cohort, only 3 of 250 (1.2%; 95% CI, 0.4%-3.5%) seroconverted, so this cohort was omitted from additional analysis.

Table 3 also shows the proportions of those who seroconverted as an indicator of the variation in risk of infection. In the community cohort, 13% seroconverted vs 29% in the military and 7% in the hospital staff cohort. Community participants aged 20 through 24 years were at higher risk than older participants with 21% of those in community and 24% of those in the military cohorts seroconverting vs 8% of those 60 years or older in the community cohort. Furthermore, 44% of those aged 15 through 19 years in the military cohort seroconverted. No discernible effect from prior seasonal influenza vaccination existed except for the military cohort, for which 37% of unvaccinated participants seroconverted vs 19% of those vaccinated. Participants with higher baseline titers had lower seroconversion rates—13% of military participants with titers of 40 or higher seroconverted vs 32% with titers lower than 10. Seroconversion data were available for 223 participants residing in the 106 households in the community cohort. Twenty-nine percent (10/34; 95% CI, 17%-46%) of those living with another household member who was known to have seroconverted vs 12% (23/189; 95% CI, 8%-18%) of those living in households in which no one else had seroconverted and 13% (65/504; 95% CI, 10%-16%) in other community participants for whom seroconversion data for other household members were not available had seroconversion. Because there was no significant difference in seroconversion rates for the latter 2 groups ($P = .79$), these were combined during multivariate analysis.

On multivariate analysis (TABLE 4), having another household member who seroconverted remained associated with

Table 3. Factors Associated With Seroconversion by Cohorts

	Participants With Seroconversion by Cohort					
	Community Members		Military Personnel		Hospital Staff	
	No./Total	% (95% CI)	No./Total	% (95% CI)	No./Total	% (95% CI)
Detection of seroconversion by blood draw						
Baseline to second blood draw	70/621	11 (9-14)	254/920	28 (25-31)	16/501	3 (2-5)
Second to third	16/584	3 (2-4)	21/636	3 (2-5)	12/432	3 (2-5)
Baseline to third	83/690	12 (10-15)	223/776	29 (26-32)	32/468	7 (5-9)
Ever	98/727	13 (11-16)	312/1060	29 (27-32)	35/537	7 (5-9)
Age, y[a]						
15-19			115/259	44 (38-50)		
20-24	16/78	21 (13-31)	96/399	24 (20-28)	6/104	6 (3-12)
25-29	5/50	10 (4-21)	11/75	15 (8-24)	9/123	7 (4-13)
30-39	18/132	14 (9-21)	1/37	3 (0-14)	7/157	4 (2-9)
40-49	43/267	16 (12-21)	0/28	0 (0-12)	10/95	11 (6-18)
50-59	12/147	8 (5-14)	1/11	9 (2-38)	2/51	4 (1-13)
≥60	4/53	8 (3-18)	1/4	25 (5-70)	1/7	14 (3-51)
Sex[a]						
Male	45/295	15 (12-20)	308/1028	30 (27-33)	5/90	6 (2-12)
Female	53/432	12 (10-16)	4/32	13 (5-28)	30/447	7 (5-9)
Seasonal influenza vaccine[a]						
No	87/659	13 (11-16)	227/616	37 (33-41)	1/50	2 (0-10)
Yes	11/68	16 (9-27)	85/444	19 (16-23)	34/487	7 (5-10)
Baseline titers[a]						
<10	93/631	15 (12-18)	252/799	32 (28-35)	27/340	8 (6-11)
10	2/48	4 (1-14)	30/91	33 (24-43)	8/126	6 (3-12)
20	3/27	11 (4-28)	17/71	24 (16-35)	0/36	0 (0-10)
≥40	0/21	0 (0-15)	13/99	13 (8-21)	0/35	0 (0-10)
Other household member[a,b]						
≥1	10/34	29 (17-46)				
No one else	23/189	12 (8-18)				
Other	65/504	13 (10-16)				

Abbreviation: CI, confidence interval.
[a] Numerator is individuals who had ever seroconverted; denominator is individuals who had at least 1 follow-up sample (second, third, or both samples).
[b] Other household member seroconverted: at least 1 other household member with seroconversion, no one else in household with seroconversion, and other combinations (no other household member in the study or other members in the study but seroconversion data not available).

a higher likelihood of infection (adjusted OR, 3.32; 95% CI, 1.50-7.33). The proportion within the unit who had seroconverted was associated with increased risk of infection in the military (adjusted OR, 1.42; 95% CI, 1.27-1.59) but not among hospital staff. After adjusting for infections in the same military unit, vaccination and sex were no longer significant, but older age remained significantly protective (adjusted OR, 0.42 per 10 years; 95% CI, 0.27-0.65), similar to the community cohort (adjusted OR, 0.77 per 10 years; 95% CI, 0.64-0.93). Higher baseline titers had lower likelihood of seroconversion in the community (adjusted OR, 0.48; 95% CI, 0.27-0.85), hospital staff (adjusted OR, 0.50; 95% CI, 0.26-0.93), and military cohorts (adjusted OR, 0.71; 95% CI, 0.61-0.81).

During the study period, acute respiratory and febrile respiratory illness episodes were more common for individuals who seroconverted. In community participants, 73% (72/98; 95% CI, 64%-81%) of those who had seroconverted reported 1 or more acute respiratory illness episodes vs 43% (269/629; 95% CI, 39%-47%) of those who had not ($P<.001$), and 44% (43/98; 95% CI, 34%-54%) of those who had seroconverted had febrile respiratory illness episodes vs 9% (56/629; 95% CI, 7%-11%) of those who had not ($P<.001$). Among hospital staff, 69% (24/35; 95% CI, 52%-81%) of those who had seroconverted had acute respiratory illness vs 15% (75/502; 95% CI, 12%-18%) of those who had not ($P<.001$), and 51% (18/35; 95% CI, 36%-67%) of those who had seroconverted had febrile respiratory illness vs 8% (41/502; 95% CI, 6%-11%) of those who had not ($P<.001$). The military cohort reported lower acute respiratory illness and febrile respiratory illness rates: 31% (98/312; 95% CI, 27%-37%) of those who had seroconverted had acute respiratory illness vs 24% (181/748; 95% CI, 21%-27%) of those who had not ($P=.02$), and 16% (50/312; 95% CI, 12%-21%) of those who had seroconverted had febrile respiratory illness vs 7% (56/748; 95% CI, 6%-10%) of those who had not ($P<.001$).

COMMENT

To our knowledge, this is the first cohort study designed to estimate the extent of infection with 2009 influenza A(H1N1) using serological assays. Our study shows that at the end of the first epidemic wave in Singapore a substantial proportion of the Singapore adult population lack antibodies to the novel strain, with only 13% of the community cohort having serological evidence of infection. This infection rate estimate is compatible with the 11% clinical attack rate for Singapore estimated from influenza-like illness reporting[7] and was fairly similar to estimates of adult incidence from a cross-sectional serological study conducted after the first epidemic wave of 2009 influenza A(H1N1) in the United Kingdom.[14]

Our study also shows the variation in infection risks, with younger age groups and military personnel having much higher infection rates. The lower infection rates in older participants corroborate other epidemiological observations.[14,15] Because there was no 15- to 19-year age group in the community cohort, we are unable to conclude whether higher infection rates in the military were due to the younger age or increased transmission, although historical pandemic data[16] and the strong association between infection risk and level of intraunit infections in our study

Table 4. Univariate and Multivariate Analysis for Factors Associated With Seroconversion in Community, Military, and Hospital Staff

	Crude OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Community				
Age per 10 y	0.81 (0.67-0.97)	.02	0.77 (0.64-0.93)	.007
Female sex	0.78 (0.51-1.19)	.25		
Had seasonal influenza vaccine	1.27 (0.64-2.51)	.50		
Baseline titer[a]	0.54 (0.31-0.94)	.03	0.48 (0.27-0.85)	.01
Other household member[b]	2.86 (1.33-6.19)	.007	3.32 (1.50-7.33)	.003
Military				
Age per 10 y	0.25 (0.15-0.41)	<.001	0.42 (0.27-0.65)	<.001
Female sex	0.33 (0.12-0.96)	.04		
Had seasonal influenza vaccine	0.41 (0.30-0.54)	<.001		
Baseline titer[a]	0.76 (0.66-0.87)	<.001	0.71 (0.61-0.81)	<.001
Proportion in unit (per 10%)[c]	1.56 (1.40-1.72)	<.001	1.42 (1.27-1.59)	<.001
Hospital				
Age per 10 y	1.06 (0.76-1.46)	.74		
Female sex	1.22 (0.46-3.24)	.69		
Had seasonal influenza vaccine	3.68 (0.49-27.46)	.20		
Baseline titer[a]	0.50 (0.26-0.93)	.03	0.50 (0.26-0.93)	.03
Proportion in unit per 10%[c]	1.24 (0.83-1.85)	.30		

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] For every unit increase in baseline titer, for which the integer values 0 to 8 denote titers of <10, 10, 20, 40, 80, 160, 320, 640, and 1280 or more, respectively.
[b] Had at least 1 other household member who seroconverted compared with all other community participants (including those from households for which no one else seroconverted, no other household member in the study, or other members in the study but seroconversion data not available).
[c] Proportion in unit who seroconverted.

point to greater transmission intensity in military populations. This suggests that special preventive measures in military subpopulations may be justified in the event of influenza epidemics. The increased risk of infection for community participants from households in which at least 1 other member seroconverted was expected, although it was not possible to determine the direction of transmission in this study.

In contrast, hospital staff and the long-term care facilities cohorts had lower infection rates. Besides the high baseline titers, hospital staff may also have been protected at work because of intense patient and visitor screening, use of personal protective equipment, and other infection control measures deployed during the epidemic.[17,18] Such combination strategies may help prevent influenza transmission, although it is difficult to attribute the specific effect of these interventions without control groups. Staff and residents of the long-term care facilities may likewise have been protected by similar measures, but other factors such as reduced host susceptibility in the older age groups should be considered, for as others have found, long-term care facilities were largely spared from 2009 influenza A(H1N1) outbreaks.[19] Because large segments of these populations lacked antibodies after the initial epidemic wave, outbreaks might occur in subsequent epidemic waves. Likewise, only 13% of the community cohort seroconverted, which supports the case for targeted vaccination in populations for which protection is desired.

In both the community cohort and hospital staff, about half the participants who seroconverted reported a febrile respiratory illness episode. This is comparable with estimates of influenza-like illness proportions among serologically confirmed influenza cases from seasonal influenza studies.[20,21] Febrile respiratory illness episodes were less common among nonseroconverters, showing that febrile respiratory illness is reasonably specific (but not very sensitive) for influenza during epidemics.[22] The large number of community participants with acute respiratory illness episodes who did not seroconvert may have had other infections; rhinovirus circulates throughout the year and is the most common identifiable cause of acute respiratory illness in Singapore.[23,24] There were proportionately fewer febrile respiratory illness episodes in military personnel possibly due to underreporting for which illness data were based solely on self-administered questionnaires.

Using serological cohorts is one of the best ways to estimate infection rates, particularly for large outbreaks such as 2009 influenza A(H1N1) for which laboratory confirmation cannot be performed for most cases. Our cohort study demonstrates that those with higher baseline titers have significantly lower infection rates, perhaps indicative of protection against 2009 influenza A(H1N1) infection. Our study also suggests that baseline circulating antibodies to 2009 influenza A(H1N1) exist in individuals without clinical evidence of prior infection (Table 2). Baseline antibody titers were marginally higher in vaccinated hospital staff, compatible with findings that 12% to 22% of adults experienced a 4-fold or greater increase in antibody titers to 2009 influenza A(H1N1) after seasonal influenza vaccination.[25] Our findings on age-specific prevalence of baseline antibodies are similar to those from China where only 1.7% of adults (serum samples collected July-August 2008) had preexisting antibody titers of at least 40 to 2009 influenza A(H1N1) on hemagglutination inhibition assay, with even lower responses in those 60 years or older.[26] In contrast, Hancock et al[25] found that baseline antibodies were more prevalent in older adults in the United States, suggesting that further studies are needed to understand whether the discrepant observations are due to seasonal H1N1 vaccination,[26] exposure to influenza, or other community-specific factors. Notably, in community participants aged 65 years or older (for whom vaccination is recommended), only 11% reported ever having received influenza vaccination. This corroborates previous estimates that influenza vaccine uptake in Singapore remains low.[27]

One limitation of our study is the lack of a pediatric population due to the difficulty in obtaining serial blood specimens in this age group; cross-sectional surveys using residual samples may be more feasible for estimating childhood infection rates. Furthermore, our community cohort may not be truly representative of the Singapore population because it largely comprised healthy volunteers. Although these factors preclude us from determining the actual infection rate in Singapore, our study allows us to refine estimates on the numbers at risk, obtain better case fatality rate estimates in adult age groups, and inform policy on vaccination. Finally, apart from the community cohort, the baseline collection started after influenza 2009 A(H1N1) had begun to circulate, albeit at low levels. However, subanalysis of the military and hospital cohorts found no evidence of higher baseline titers in participants whose baseline samples were collected later.

In conclusion, our study shows wide variation in serologically determined infection rates by cohorts and age groups, suggesting that context-specific risks of infection need to be taken into account and that interventions need to be tailored to the population at risk. Although it appears that a large proportion of the Singapore adult population remain susceptible to the 2009 influenza A(H1N1) virus after the first epidemic wave, for a significant second wave to occur, a sufficient number of susceptible children may also be required for efficient transmission. These and other factors will need to be considered in the determination of optimal pandemic vaccination strategies for influenza A(H1N1).[28]

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Obtained funding: Chen, Lee, Lim, R. T. P. Lin, Yap, B. H. Tan, Loh, Chow.

Administrative, technical, or material support: Chen, Lee, Lim, R. T. P. Lin, Yap, C. Lin, Laurie, L. W. L. Tan, B. H. Tan, Loh, Shaw, Durrant, Chow, Kelso, Chia.

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Financial Disclosures: Dr Cook reported that he has received research funding from the National University of Singapore. Dr Lee reported that he has received unrelated research funding from GlaxoSmithKline. No other financial disclosures were reported.

Funding/Support: This project was funded by grant NMRC/H1N1O/005/2009 from the National Medical Research Council of Singapore. The Melbourne World Health Organization Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health and Ageing; the Centre has collaborative projects with vaccine companies unrelated to this study. The military cohort study was funded by the Ministry of Defence, Singapore.

Role of the Sponsor: None of the sponsors participated in the design and conduct of the study, in the collection, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

Additional Contributions: We thank Pei Ling Loh, BSc, Nataline Tang, BSc, and Dollyn Quek, BSc, of the National Public Health Laboratory in Singapore, and Baldev Singh, AN, Caroline Lee, BSc, Jessie Tan, BSc, Rachelle Goh, RN, and Elaine Pek, MHSM, from the Infectious Disease Research Centre at Tan Tock Seng Hospital; Angela Cheong, BSc, from the National University of Singapore; Joshua Tay, MBBS, and Christine Gao, BSc, from the Singapore Armed Forces; and Janet Chew, BSc, from the DSO, National Laboratories, Singapore, for their contributions to this study. We thank the Communicable Diseases Division, Ministry of Health, Singapore, for contributing national surveillance data and other support during the conduct of the study. All individuals acknowledged here performed their roles as part of their regular duties and were not additionally compensated for their contributions.

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