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Seroprevalence of SARS Coronavirus Among Residents Near a Hospital with a Nosocomial Outbreak

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Background/Purpose: An epidemic of severe acute respiratory syndrome (SARS) occurred in Taiwan from April to July 2003. A nosocomial outbreak of SARS occurred at Kaohsiung Chang Gung Memorial Hospital (CGMH) in May 2003. The purpose of our study was to survey the prevalence of the SARS coronavirus (CoV) in a community adjacent to Kaohsiung CGMH and collect demographic data, including basic information about health status, household, and possible risk factors for SARS-CoV infection.

Methods: We randomly recruited 1030 persons living in three precincts adjacent to Kaohsiung CGMH. For all subjects, we collected demographic data and measured the seroprevalence of the SARS-CoV with an enzyme-linked immunosorbent assay (ELISA) and an immunofluorescence assay (IFA).

Results: The ELISA was seropositive for 124 of 1030 participants (12%). The more sensitive and specific IFA confirmed SARS in only two cases (0.19%). Both confirmed cases were under 19 years of age and had no known SARS-related risk factors.

Conclusion: There was low post-epidemic seroprevalence of SARS-CoV in a community adjacent to a hospital which had a nosocomial SARS outbreak. The SARS outbreak in Taiwan was primarily limited to hospital settings. [*J Formos Med Assoc* 2008;107(11):885–891]

Key Words: community survey, nosocomial infections, SARS-CoV, seroprevalence

Severe acute respiratory syndrome (SARS), caused by the SARS coronavirus (CoV), is an emergent disease that was first reported in the Guangdong Province of China in November 2002.^{1–3} It spread rapidly to Vietnam, Canada, and Hong Kong.^{4–6} Within months of its emergence in China, it had affected more than 8000 people and caused 774 deaths in 26 countries on five continents.^{7,8}

International air-travel routes and the presence of densely populated urban areas, especially in Asia, facilitated the rapid worldwide spread of the SARS-CoV.^{9,10}

In mid-March 2003, several SARS cases were reported in Taiwan, presumably because of its extensive business ties with Hong Kong and China.¹¹ In April 2003, a severe nosocomial outbreak of

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SARS occurred at Taipei Municipal Ho-Ping Hospital (in northern Taiwan), where an unrecognized index patient exposed multiple patients, visitors, and health care workers.¹² This outbreak resulted in more than 10 fatalities and led health authorities near this hospital to implement control procedures.¹² Nonetheless, in early May 2003, the SARS infection had spread to multiple cities and regions of Taiwan, and there was a large outbreak at Kaohsiung Chang Gung Memorial Hospital (CGMH) in southern Taiwan. Kaohsiung CGMH temporarily discontinued all emergency and hospital services. By the end of the SARS epidemic in Taiwan (July 2003), there were 347 confirmed cases and 37 confirmed deaths.¹³

Because of its high infectivity and hazard to global health, scientists have attempted to develop reliable tests, find effective treatment protocols and determine the key epidemiologic parameters that affect the spread of SARS-CoV.² Previously, we found that some SARS patients in Taiwan had no apparent contact histories or travel histories to epidemic areas, suggesting that there might have been a sporadic community spread, especially in Taipei City and in Kaohsiung City.

In the present study, we used two serologic methods to survey the prevalence of the SARS-CoV in a community adjacent to Kaohsiung CGMH where there was a nosocomial outbreak. We also collected demographic data, including

basic information about health status, household, and possible risk factors for SARS-CoV infection.

Methods

Study area, study design, collection of serologic data

We conducted a survey of SARS-CoV seroprevalence from August 2003 (1 month after the epidemic had ended) to December 2003 in the San-Ming District (population: 361,330) of Kaohsiung City (population: 1,509,699). Chang Gung Memorial Hospital (CGMH; 2464 beds), which experienced a nosocomial outbreak of SARS in May 2003, is adjacent to the San-Ming District (Figure). We selected three precincts of this district, all of which are within three city blocks (approximately 500 m) of the hospital, for serologic tests and questionnaire surveys. We conducted age- and gender-stratified sampling using household registration records. All study subjects provided written informed consent for participation. The Institutional Review Board of CGMH approved this study.

Each participant completed a self-administered questionnaire that collected basic demographic data including: residential area; occupation (health care worker or not); history of chronic diseases during the epidemic; quarantine during

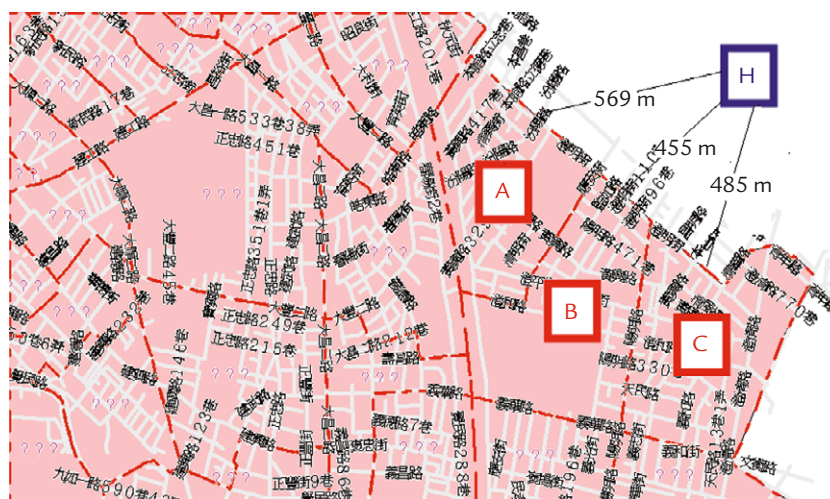


Figure. San-Ming District of Kaohsiung City (1:12,000). H=Chang Gung Memorial Hospital; A, B, C=precincts from which residents were sampled.

the epidemic; intrafamilial or outside contact with SARS cases during the epidemic; travel history in the previous 6 months; classmates or neighbors with SARS during the epidemic; public conveyance used in the previous 6 months; illnesses during the previous 3 months; and preventive measures used (such as a mask). People who used masks were asked to specify the mask type (paper, surgical, or N95). We defined contacts of SARS cases as persons who shared meals, a residence, a hospital room, or a transportation vehicle with a suspected SARS patient or as persons who visited a suspected SARS patient within 14 days before the patient's onset of symptoms. We also considered persons who had potential contact with the secretions of a SARS patient during the patient's treatment or care as having had close contact.

Laboratory methods for the detection of SARS-CoV antibody

After interviewing each subject, we collected a 5 mL blood sample for SARS antibody detection, refrigerated it at 4 °C, and screened it for the SARS-CoV antibody using whole viral lysate enzyme-linked immunosorbent assay (ELISA; Beijing Huada GBI Biotechnology Co. Ltd., Beijing, China).^{14,15} If the ELISA test was positive, we used the indirect immunofluorescence assay (IFA; Euroimmun Co, Lübeck, Germany) to retest for the presence of the SARS-CoV antibody.¹⁶⁻¹⁸ We performed an IgG test for the SARS-CoV by an indirect ELISA test that used the lysate of whole SARS-CoV as the coated antigen.¹⁷ The cutoff value for a positive IgG test by ELISA was 0.13 absorbance units above the negative control.

We performed IFA testing using a diluted serum specimen that reacted against SARS-CoV-infected Vero E6 cells and non-infected cells.^{17,18} First, we placed 25 µL of serial diluted serum (starting from 1:10) onto each well of the slide and incubated them for 30 minutes at room temperature. After washing for 5 minutes twice with phosphate-buffered saline (PBS), we added 20 µL of diluted fluorescein-labeled anti-human globulin to each well, and incubated them for 30 minutes at room temperature. Then we washed

the slides twice with PBS and observed samples under a fluorescence microscope. The cutoff value for a positive IgG test was 1:10.

Statistical analysis

Confidence intervals were calculated using binomial proportion, a function built in SAS PROC FREQ in SAS version 9.0 (SAS Institute Inc., Cary, NC, USA).

Results

We randomly selected 2854 residents using an age- and gender-stratified sampling method from the household registration record and attempted to contact each person by telephone. We excluded subjects who were not living in Kaohsiung City during the epidemic. The most frequent reasons given for refusal to participate were "tired of being interviewed" and reluctance to disclose personal information for fear of discrimination. We successfully interviewed and obtained blood samples from 1030 residents (36.1%). None of the subjects had a clinical diagnosis of SARS or had been quarantined during the epidemic. A total of 258 (25%) subjects had underlying diseases, most of which (249/258; 96.5%) were mild to moderate. We defined "mild to moderate underlying diseases" (e.g. allergic diseases, atopic dermatitis, hepatitis B) as diseases that were not life-threatening, or that did not result in immunodeficiency.

We analyzed factors associated with SARS-CoV infection and stratified the results by age (Table 1). Forty subjects reported contact with quarantined person(s). In most cases (33/40; 82.5%), they were students whose classmates had family members working in Kaohsiung CGMH during the epidemic. The two patients with SARS-CoV seropositivity (as confirmed by IFA) reported no contact with quarantined persons during the epidemic. None of the quarantined persons who had contact with our subjects were diagnosed as SARS-probable cases during the epidemic. Analysis of other selected factors associated with SARS revealed that only 17.5% (180/1030) of people used public

Table 1. Age-specific characteristics, potential risks and protective factors among residents of three precincts adjacent to a nosocomial SARS outbreak ($n = 1030$)

Factors potentially associated with SARS-CoV infection	n (%)	95% CI
Hospital related (visits to a hospital during epidemic*)	139 (13.5)	11.47–15.73
< 10 yr	10 (7.19)	3.50–12.83
10–19 yr	25 (17.99)	11.99–25.39
20–39 yr	23 (16.55)	10.79–23.79
40–49 yr	34 (24.46)	17.57–32.47
≥ 50 yr	47 (33.81)	26.01–42.32
Community related		
Contact with a quarantined person during epidemic	40 (3.9)	2.79–5.25
< 10 yr	0 (0)	0–0
10–19 yr	36 (90.00)	76.34–97.21
20–39 yr	2 (5.00)	0.61–16.92
40–49 yr	1 (2.50)	0.06–13.16
≥ 50 yr	1 (2.50)	0.06–13.16
History of traveling to SARS-affected countries [†] in previous 6 mo	8 (0.77)	0.33–1.52
Types of transportation used during the epidemic		
None mentioned	2 (0.19)	0.02–0.70
None	117 (11.36)	9.42–13.30
Type of transportation	911 (88.45)	86.49–90.40
Private only (cars or motorcycles)	731 (80.24)	77.66–82.83
< 10 yr	166 (22.71)	19.67–25.75
10–19 yr	132 (18.06)	15.27–20.85
20–39 yr	129 (17.65)	14.88–20.41
40–49 yr	133 (18.19)	15.40–20.99
≥ 50 yr	171 (23.39)	20.32–26.46
Public only (taxis, trains or buses)	159 (17.45)	14.99–19.92
< 10 yr	0 (0)	0–0
10–19 yr	135 (84.91)	78.38–90.08
20–39 yr	6 (3.77)	1.40–8.03
40–49 yr	3 (1.89)	0.39–5.41
≥ 50 yr	15 (9.43)	5.38–15.08
Private and public conveyances used	21 (2.31)	1.43–3.50
< 10 yr	0 (0)	0–0
10–19 yr	0 (0)	0–0
20–39 yr	7 (33.33)	14.59–56.97
40–49 yr	3 (14.29)	3.05–36.34
≥ 50 yr	11 (52.38)	29.78–74.29
Masks used when working or going out during the epidemic	607 (58.9)	55.86–61.96
< 10 yr	104 (17.13)	14.22–20.37
10–19 yr	180 (29.65)	26.04–33.46
20–39 yr	102 (16.80)	13.91–20.02
40–49 yr	94 (15.49)	12.70–18.61
≥ 50 yr	127 (20.92)	17.75–24.38

*Hospitals that had an outbreak during the epidemic included Kaohsiung Medical University Hospital, Kaohsiung Chang Gung Memorial Hospital, Taipei Municipal Ho-Ping Hospital and National Taiwan University Hospital; [†]China, Hong Kong, Canada, Vietnam and Singapore.

Table 2. Age-specific seropositivity rates after the SARS epidemic among residents of three precincts adjacent to a nosocomial SARS outbreak

Age (yr)	Cases tested		ELISA-seropositive cases		IFA-seropositive cases	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Total	1030	–	124 (12.04)	10.11–14.18	2 (0.19)	0.02–0.70
<10	202 (19.61)	17.23–22.17	67 (33.17)	26.72–40.12	1 (0.49)	0.01–2.73
10–19	334 (32.43)	29.57–35.38	54 (16.17)	12.39–20.56	1 (0.30)	0.01–1.66
20–29	61 (5.92)	4.56–7.54	2 (3.28)	0.40–11.35	0	0
30–39	78 (7.57)	6.03–9.36	1 (1.28)	0.03–6.94	0	0
40–49	142 (13.79)	11.74–16.04	0	0	0	0
≥50	213 (20.68)	18.24–23.28	0	0	0	0

ELISA = enzyme-linked immunosorbent assay; IFA = indirect fluorescence assay.

conveyance (i.e. taxis, trains or buses), most of whom (135/180; 75.0%) were under 19 years of age. Of the 139 persons who had visited hospitals where outbreaks occurred during the epidemic, 58.3% (81/139) were older than 40 years. Most (607/1030; 58.9%) subjects reported using masks as a preventive measure during the epidemic. The most common types of masks were paper (263/607; 43.3%) or surgical masks (256/607; 42.2%). Only a small number (8/1030; 0.8%) of subjects had traveled to SARS-affected countries (China, Hong Kong, Canada, Vietnam, or Singapore) in the 6 months before this investigation.

The age-specific SARS-CoV seropositivity rates after the epidemic are shown in Table 2. Of the 124 persons with a seropositive ELISA, nearly 98% (121/124) were younger than 19 years of age. We used the IFA method to confirm the presence of SARS-CoV in all subjects who had a positive ELISA result. IFA confirmed SARS-CoV in two of the 124 cases, both of whom were under 19 years of age. We found no SARS-related risk profiles or signs of respiratory tract infection in these two patients during the 3 months after the epidemic.

Discussion

Main findings of this study

This study provides important information about the potential for the spread of SARS from a hospital to a local community. The Taiwanese community

we studied was next to a hospital that had a nosocomial outbreak. Only 0.19% (2/1030) of study subjects in the community had positive ELISA and IFA results for SARS-CoV. The two infected patients were asymptomatic, had no history of contact with quarantined individuals, no history of travel to epidemic areas and did not visit the hospital where there was a nosocomial outbreak. Our results indicate very limited spread of the SARS-CoV into an adjacent community following a nosocomial outbreak.

What is already known on this topic

A previous post-epidemic serologic survey in Hong Kong showed a low SARS-CoV seropositivity rate (0.19%; 2/1068) in 1068 asymptomatic close contacts of SARS patients.¹⁵ Those results are consistent with our findings. Another SARS seroprevalence study of 574 general practitioners in Hong Kong found 0% infection rate of SARS-CoV in the at-risk group.¹⁹

Even in hospital settings, however, the seroprevalence of SARS in health care workers is still not high. For example, a seroprevalence study of 193 emergency department workers exposed to SARS in a Taiwanese medical center found that the incidence of SARS-CoV infection was only 4.7% (9/193).¹⁸ Taken together, these previous studies indicated that although transmission of SARS-CoV occurs primarily in hospital settings, transmission in hospital settings and in the community are generally not serious. Although asymptomatic and

mild SARS infections have been documented in other countries, these seem to be uncommon and do not appear to extend the chain of infection.²⁰

What this study adds

Our analysis of the risk factors for SARS-CoV transmission indicated that 13.5% of subjects had visited a hospital where a SARS outbreak occurred, 0.8% had traveled to affected countries, and 3.9% had contact with a quarantined individual during the epidemic. Most subjects (58.9%) reported having used masks as preventive measures and having infrequently (17.5%) used public conveyances. A previous study showed that use of masks significantly lowered the risk of SARS-CoV transmission.²¹ The low prevalence of SARS-CoV in our study population may be because SARS-CoV is transmitted via direct or indirect contact of the mucous membrane with infectious respiratory droplets.²² However, our results do not exclude the possibility of a small subclinical asymptomatic infection of SARS-CoV in this community, as has been previously observed in Hong Kong.²³

Study limitations

Three tests are used to diagnose infection with the SARS-CoV: ELISA, IFA and RT-PCR.¹⁶ We used the simple ELISA test for all 1030 subjects and the IFA for subjects who had positive ELISA results. The IFA is highly sensitive and specific, but is labor intensive. Antibody assays based on virus-infected cells or whole viral lysates (such as the ELISA test) can produce false positives from closely related viruses of the Coronaviridae.²⁴ In particular, because "common cold"-associated coronavirus infections are highly prevalent, whole virus-based assays, which have low specificity, cannot be used for the diagnosis of SARS-CoV.¹⁹ In our case, although 124 subjects were seropositive based on ELISA, we confirmed SARS-CoV infection in only two patients by the more precise and specific IFA.

Similar results have been reported in a seroprevalence study of SARS infection of general practitioners in Hong Kong, in which 5.1% tested positive by ELISA but none tested positive by

IFA.¹⁷ IFA is an infected cell-based test for anti-SARS IgG antibody that provides high sensitivity and specificity.²⁴ A recent study by Chan et al showed that the sensitivity and specificity of the IFA were both 100%.²⁴

Conclusion

This study found low post-epidemic seroprevalence of SARS-CoV in a Taiwanese community that is adjacent to a hospital where a nosocomial outbreak occurred. Our findings support previous reports that the April–July 2003 SARS outbreak in Taiwan was primarily limited to hospital settings, with only rare transmission by casual and social contacts.²⁰ Although asymptomatic carriers or subclinical infections can occur in the community, the present study and other recent studies²⁵ suggest that these are unlikely to be a source of SARS reemergence. The results of the present study will prove important for the development and implementation of policies for the control of SARS and possibly for the control of other infectious diseases which have similar modes of transmission.

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