Leung, GM; Lim, WW; Ho, LM; Lam, TH; Ghani, AC; Donnelly, CA; Fraser, C; Riley, S; Ferguson, NM; Anderson, RM; Hedley, AJ (2006) Seroprevalence of IgG antibodies to SARS-coronavirus in asymptomatic or subclinical population groups. Epidemiology and infection, 134 (2). pp. 211-21. ISSN 0950-2688

Downloaded from: http://researchonline.lshtm.ac.uk/11565/
SYSTEMATIC REVIEW
Seroprevalence of IgG antibodies to SARS-coronavirus
in asymptomatic or subclinical population groups

G. M. LEUNG1,2*, W. W. LIM3, L.-M. HO1, T.-H. LAM1, A. C. GHANI4,
C. A. DONNELLY4, C. FRASER4, S. RILEY4, N. M. FERGUSON4,
R. M. ANDERSON1 AND A. J. HEDLEY1

1 Department of Community Medicine, University of Hong Kong, Pokfulam, Hong Kong, China
2 Takemi Program, Harvard School of Public Health, Boston, MA, USA
3 Government Virus Unit, Public Health Laboratory Centre, Shek Kip Mei, Kowloon, Hong Kong, China
4 Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College, University of London,
  St Mary’s Campus, Norfolk Place, London, UK

(Accepted 21 April 2005, first published online 22 July 2005)

SUMMARY
We systematically reviewed the current understanding of human population immunity against
SARS-CoV in different groups, settings and geography. Our meta-analysis, which included all
identified studies except those on wild animal handlers, yielded an overall seroprevalence of
0.10% [95% confidence interval (CI) 0.02–0.18]. Health-care workers and others who had close
contact with SARS patients had a slightly higher degree of seroconversion (0.23%, 95% CI
0.02–0.45) compared to healthy blood donors, others from the general community or non-SARS
patients recruited from the health-care setting (0.16%, 95% CI 0–0.37). When analysed by the two
broad classes of testing procedures, it is clear that serial confirmatory test protocols resulted in a
much lower estimate (0.050%, 95% CI 0–0.15) than single test protocols (0.20%, 95% CI
0.06–0.34). Potential epidemiological and laboratory pitfalls are also discussed as they may
give rise to false or inconsistent results in measuring the seroprevalence of IgG antibodies
to SARS-CoV.

INTRODUCTION
Major outstanding questions about severe acute respiratory syndrome (SARS) remain in order to com-
plete the agent–vector–host epidemiological triangle (Fig. 1). Is there a significant human reservoir of
SARS-coronavirus (CoV) from either the 2003 epidemic or perhaps through previous but undetected
circulation of the virus? Were there a limited number of susceptibles within the population before the

* Author for correspondence: Dr G.M. Leung, Department of
Community Medicine, Faculty of Medicine, University of Hong
Kong, 21 Sassoon Road, Pokfulam, Hong Kong, China.
(Email: gmleung@hkucc.hk)
systematic study of the seroepidemiology of SARS-CoV in different population groups.

Epidemiological and laboratory methods for the study of seroprevalence

The study of population immunity and prevalence of past infection is typically based on systematic random sampling from the general population with appropriate stratification, or on different groups with a priori varying degrees of risk for infection.

Systematic adherence to the basic epidemiological principles of unbiased, random sampling is important. The sampling frame and size must be defined clearly and in the case of special surveys the response and participation rate is also important. Together, these components determine the validity and precision of the estimates of seroprevalence ratios. The numerator of the ratio includes those who test positive based on a series of pre-defined immunological tests, each with a particular threshold of serological titre to immunoglobulin (Ig) G antibodies against the agent under consideration, indicating the number of people in the sample who had been infected at some stage of their life. Because SARS is a newly emergent human disease, this also represents the extent of asymptomatic spread since the first reported human case in November 2002 in Guangdong [3]. The appropriate laboratory tests for serological diagnosis vary depending on the agent. Moreover, the sequence of different tests is important as it changes the Bayesian pre-test probability of a positive result and thus, the overall sensitivity and specificity of the particular testing protocol. Serial testing, where only positive samples on the initial test proceed to the next test, generally increases specificity but decreases sensitivity, while parallel testing where different tests are performed simultaneously has the opposite effect. For SARS-CoV, the most widely adopted methods for detection of antibodies are indirect immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays (ELISA) with cell-culture extracts from which positive screens are confirmed using standard virological neutralization tests [4]. Alternative approaches have been suggested such as ELISA-based antibody detection tests using recombinant antigens with positive screens confirmed by Western blots that use two different antigenic proteins (nucleocapsid protein and spike polypeptide) of SARS-CoV [5]. It is difficult, especially for newly emerging diseases such as SARS, to decide initially which set of laboratory techniques are optimal for antibody serosurveys. A careful comparison of these different methods against established gold standards is essential, using benchmark indices including sensitivity, specificity, the area under the receiver operating characteristic curve and likelihood ratios [6]. In addition, cross-reactivity of these assays to related microbial agents must be considered in order to achieve specificity and reduce false positives to a minimum.

Serosurveys for SARS-CoV IgG antibodies

To identify relevant serosurveys for SARS-CoV antibodies, we searched MEDLINE for articles published between January 2003 and July 2004 using combinations of the MeSH terms ‘SARS virus’, ‘severe acute respiratory syndrome’, ‘seroepidemiologic studies’ and/or ‘antibodies’, and keywords ‘serosurvey’ and/or ‘seroprevalence’. We also searched relevant publications and websites of the World Health Organization (WHO), US Centers for Disease Control and Prevention (CDC) and other similar national or regional agencies of SARS-affected places to identify studies that were potentially not included in MEDLINE. We searched the bibliographies of identified studies manually and consulted with experts in the field to try and locate other reports not found through our main search strategy.

Our inclusion criteria were broad and required only reporting of seroprevalence (i.e. both numerator and denominator data) of SARS-CoV in individuals who were never diagnosed with SARS as defined by the WHO [7]. We did not place any limits on epidemiological study design, laboratory methods or language of publication. Data were abstracted from the original source publication by two independent, blinded research assistants. Potential disagreements were settled by a third researcher after independent review.

A total of 16 studies, from five SARS-affected regions (Beijing, Guangzhou, Hong Kong, Singapore...
Seroprevalence of IgG antibodies to SARS-coronavirus

There is wide variation in the reported seroprevalence of antibodies against SARS-CoV which is strongly associated with the characteristics of the subjects tested (indicating a priori risk of infection) and the laboratory methods employed to determine seroconversion.

With the exception of handlers of wild animals and market workers, the degree of asymptomatic infection was <3% for all studies (Table). We combined the results of these studies, stratified by subgroups indicating the a priori risk of infection with the timing of specimen collection classified as pre- vs. post-SARS epidemic (Fig. 2), and by laboratory testing protocols (Fig. 3). The 95% confidence intervals (CI) for the seroprevalence estimates in each study were computed using exact binomial CIs. The meta program [22] was employed to calculate weighting associated with individual studies. In calculating the standard errors, if a study had no seropositive cases (i.e. a zero numerator), a count value of 0·1 was used instead according to the usual convention, to avoid the problem of zero-count cells while minimizing the potential to inflate precision of the underlying study. The method of DerSimonian & Laird [23] was used to test for heterogeneity across studies which was significant (P < 0·001) for both sets of meta-analyses presented in Figures 2 and 3. Therefore, a random-effects model was assumed in computing the weights of the study and variances of the combined estimates. The 95% CIs for the combined estimates were calculated by normal approximation.

Our meta-analysis, which included all identified studies except those on handlers of wild animals due to the presumed zoonotic origin of SARS-CoV and the very different associated risk of infection (Fig. 3), yielded an overall seroprevalence of 0·10% (95% CI 0·02–0·18). Figure 2 shows that the summary seroprevalence estimates of the different strata follow the same gradient of a priori risk levels, the one exception being a study of stored serum from healthy Hong Kong adults in 2001 [21]. The two Chinese studies [8, 19] in the wild animal markets of Guangzhou indicated that 14·86% (95% CI 12·77–16·94) of the workers had prior exposure to SARS-CoV although none had apparently shown significant symptoms compatible with the clinical description of SARS. Health-care workers and others who had close contact with SARS patients generally had a slightly higher degree of seroconversion (0·23%, 95% CI 0·02–0·45) compared to healthy blood donors, others from the general community or non-SARS patients recruited from the health-care setting (0·16%, 95% CI 0·0–0·37). The two studies on stored serum collected prior to the 2003 epidemic [5, 21] gave very different estimates although we note that the latter actually tested against both human and animal SARS-CoV strains. When analysed by the two broad classes of testing procedures, it is clear that serial confirmatory test protocols resulted in a much lower estimate (0·050%, 95% CI 0·0–0·15) than single test protocols (0·20%, 95% CI 0·06–0·34).

Although there are considerable variations in the seropositive estimates reported, it is clear that seroconversion is extremely rare among health-care workers, close contacts of SARS patients who did not develop the disease and members of the general population, including healthy individuals and non-SARS patients. This property of SARS-CoV, perhaps reflecting the evolutionary fitness of the virus, is in stark contrast to other common respiratory agents, most notably influenza where the usual ‘iceberg’ concept of disease applies [24]. Instead the pattern of SARS infection in the community can paradoxically be represented as an inverted iceberg (Fig. 4).

The extent of seroconversion in asymptomatic individuals with a history of intense exposure to those infected with SARS, including health-care workers and close contacts of cases, should provide the upper seropositivity limit in the general population. The overall finding of the near absence of transmission resulting in asymptomatic infection and seroconversion in these high-risk groups from different countries and settings indicates that the prevailing SARS-CoV strains almost always led to clinically apparent disease. Whereas some SARS patients might have been initially admitted in order to reduce transmission to family members, virtually all (perhaps with some exceptions in children) [25] had severe disease requiring in-patient treatment, so we can infer that the 2003 epidemic infection with SARS-CoV inevitably caused severe disease requiring hospitalization.

While the results of this meta-analysis suggest that SARS-CoV was a new virus in humans with neither a close precursor nor an antigenically related virus that would have induced at least a small degree of cross-reactivity on serological testing, the study by Zheng and colleagues [21] on the stored serum of 938 healthy Hong Kong adults from a hepatitis B serosurvey in 2001 detected a positive antibody response against human SARS-CoV or animal SARS-CoV-like virus in 1·81% (95% CI 1·06–2·89) of the sample by IFA.
<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Laboratory methods</th>
<th>Time of specimen collection</th>
<th>Subjects</th>
<th>Seroprevalence (95% exact binomial confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chan et al. [9]</td>
<td>Hong Kong</td>
<td>IFA</td>
<td>March–May 2003</td>
<td>674 health-care workers in a tertiary care hospital that admitted SARS patients</td>
<td>1.59% (0.04–8.53)</td>
</tr>
<tr>
<td>Chan et al. [10]</td>
<td>Hong Kong</td>
<td>Screened by ELISA; confirmed by IFA and Western blot</td>
<td>2003 (post-SARS epidemic)</td>
<td>12020 volunteers from the general population</td>
<td>0% (0–3.50)</td>
</tr>
<tr>
<td>Chow et al. [11]</td>
<td>Singapore</td>
<td>Screened by ELISA; confirmed by neutralization tests</td>
<td>2003 (post-SARS epidemic)</td>
<td>84 asymptomatic health-care workers at a tertiary care hospital that admitted SARS patients</td>
<td>0% (0–0.98)</td>
</tr>
<tr>
<td>Gold et al. [12]</td>
<td>Toronto, Canada</td>
<td>Screened by ELISA; confirmed by Western blot and neutralization tests</td>
<td>July–September 2003</td>
<td>767 asymptomatic health-care workers at four hospitals that admitted SARS patients</td>
<td>0.0083% (0.00021–0.046)</td>
</tr>
<tr>
<td>Ho et al. [13]</td>
<td>Singapore</td>
<td>Screened by ELISA and dot-blot immunoassay; confirmed by IFA and neutralization tests</td>
<td>2003 (paired serum samples collected during the peak of the local epidemic and on average 31 days later)</td>
<td>304 asymptomatic health-care workers at a tertiary care hospital that admitted SARS patients*</td>
<td>0% (0–0.98)</td>
</tr>
<tr>
<td>Leung et al. [4]</td>
<td>Hong Kong</td>
<td>Screened by ELISA; confirmed by IFA and neutralization tests</td>
<td>October–December 2003</td>
<td>1068 close contacts of SARS cases</td>
<td>0.19% (0.02–0.67)</td>
</tr>
<tr>
<td>Li et al. [14]</td>
<td>Guangzhou, China</td>
<td>ELISA</td>
<td>2003 (post-SARS epidemic)</td>
<td>103 contacts of SARS cases</td>
<td>0% (0–2.87)</td>
</tr>
<tr>
<td>Li et al. [15]</td>
<td>Guangzhou, China</td>
<td>IFA and ELISA</td>
<td>2003 (post-SARS epidemic)</td>
<td>1060 healthy children</td>
<td>0% (0–0.28)</td>
</tr>
<tr>
<td>Liu et al. [16]</td>
<td>Beijing, China</td>
<td>ELISA</td>
<td>2003 (post-SARS epidemic)</td>
<td>197 non-SARS hospital outpatients and in-patients ≤14 years, 156 healthy primary school children, 453 non-SARS hospital outpatients and in-patients ≥18 years</td>
<td>2.03% (0.56–5.12)</td>
</tr>
<tr>
<td>Seto et al. [17]</td>
<td>Hong Kong</td>
<td>Screened by ELISA; confirmed by neutralization tests</td>
<td>2003 (post-SARS epidemic)</td>
<td>502 adult blood donors</td>
<td>0.2% (0.01–1.10)</td>
</tr>
<tr>
<td>Wang et al. [18]</td>
<td>Beijing, China</td>
<td>ELISA</td>
<td>2003 (post-SARS epidemic)</td>
<td>875 health-care workers</td>
<td>0.1% (0.0029–0.64)</td>
</tr>
</tbody>
</table>

*Subjects in this study were asymptomatic workers at a tertiary care hospital that admitted SARS patients.
and was confirmed using neutralization tests. The researchers speculated that the virus that affected these healthy seropositive individuals was antigenically closer to the isolated animal SARS-CoV-like virus [26] than human SARS-CoV, and that this might account for the asymptomatic presentation of the infected individuals who seroconverted if the early animal strains of SARS-CoV-like virus were of low pathogenicity to humans. Some suggest that zoonotic transmission from animal to human was likely to be infrequent especially given the absence of markets of wild animals and restaurants in Hong Kong. Therefore, there was little opportunity for evolutionary selective pressures to facilitate interspecies infection of the human host in Hong Kong. Moreover, the acquisition by the virus of characteristics that enhance virulence in humans was likely to be immature. Human-to-human spread was probably highly inefficient as the virus might not have adapted in its new host. Together, these reasons were postulated to explain why only a few persons became infected and why they were likely to have been asymptomatic 2 years before the 2003 epidemic.

However, we hesitate in subscribing to this line of reasoning. First, it is important to clarify that this hypothesis is different to the presumed asymptomatic infection observed in Guangdong animal traders, especially in those who handled masked palm civets with an overall seropositivity rate of 72.7% (95% CI 49.8–89.3) [8]. Frequent zoonotic challenges in this group probably gave rise to the high asymptomatic seroconversion rate at a time when the SARS-CoV animal strains had not yet evolved into a highly pathogenic variant. Over time, sustained human exposure to the presumed animal reservoir(s) of SARS-CoV in wild animal farms and markets of southern China eventually resulted in multiple introductions of a moderately transmissible [2, 27] form of the virus into the human population [3, 28, 29] that led to the massive global outbreak. Second, it would be helpful to know how the antibody responses in 17 out of 938 subjects who seroconverted [21] were apportioned between human vs. animal SARS-CoV strains respectively. Third, and most importantly, if animal-to-human transmission was present in 2001 that led to a 1 in 55 chance of being asymptotically infected in the general population, why has this observation not been repeated in other serosurveys since? We suggest that this outlier seroprevalence estimate will remain unexplained until the study is replicated on other stored blood samples in Hong Kong and elsewhere.

**Table 1:** Seroprevalence of IgG antibodies to SARS-coronavirus

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Methodology</th>
<th>Timeframe</th>
<th>Subjects</th>
<th>Seropositivity Rate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woo et al. [5]</td>
<td>Hong Kong</td>
<td>Screened by ELISA; confirmed by two separate Western blots</td>
<td>March–May 2003 0.76% (0–1.13)</td>
<td>106 cases</td>
<td>0% (0–1.99)</td>
</tr>
<tr>
<td>Yu et al. [20]</td>
<td>Hong Kong</td>
<td>Screened by IFA; confirmed by IFA</td>
<td>June–July 2003</td>
<td>574 practitioners</td>
<td>0% (0–0.52)</td>
</tr>
<tr>
<td>Zheng et al. [21]</td>
<td>Hong Kong</td>
<td>Screened by IFA; confirmed by neutralization tests</td>
<td>May 2001</td>
<td>938 adults</td>
<td>1.81% (1.06–2.89)</td>
</tr>
</tbody>
</table>
| Positive results defined as reactivity to either human SARS-CoV or animal SARS-CoV-like virus

* An additional 62 mildly symptomatic health-care works (some admitted and others treated as outpatients) were also included in the study, two of whom tested positive, yielding a seroprevalence of 3.23% (95% CI 0.39–11.2).
<table>
<thead>
<tr>
<th>Study</th>
<th>No. of seropositives/No. tested</th>
<th>Seroprevalence in % (95% CI)</th>
<th>% weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild animal handlers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xu et al. [19]</td>
<td>106/635</td>
<td>16.69 (13.87–19.83)</td>
<td>0.24</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>14.86 (12.77–16.94)</td>
<td>0.48</td>
</tr>
<tr>
<td>Health-care workers and close contacts of SARS patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC [8]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital workers</td>
<td>4/137</td>
<td>2.92 (0.80–7.31)</td>
<td>0.26</td>
</tr>
<tr>
<td>Public health officials</td>
<td>1/65</td>
<td>1.59 (0.04–8.53)</td>
<td>0.22</td>
</tr>
<tr>
<td>Chan et al. [9]</td>
<td>0/674</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Chow et al. [11]</td>
<td>0/84</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Gold et al. [12]</td>
<td>8/767</td>
<td>1.04 (0.45–2.04)</td>
<td>2.81</td>
</tr>
<tr>
<td>Ho et al. [13]</td>
<td>0/304</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Leung et al. [4]</td>
<td>2/1068</td>
<td>0.19 (0.02–0.67)</td>
<td>7.05</td>
</tr>
<tr>
<td>Li et al. [14]</td>
<td>0/103</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Wang et al. [18]</td>
<td>29/1127</td>
<td>2.57 (1.73–3.67)</td>
<td>1.94</td>
</tr>
<tr>
<td>Woo et al. [5]</td>
<td>0/33</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Yu et al. [20]</td>
<td>0/574</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Subtotal</td>
<td>1.96</td>
<td>0.38 (0.03–6.46)</td>
<td>0.38</td>
</tr>
<tr>
<td>Health individuals from the general community or non-SARS patients</td>
<td></td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>CDC [8]</td>
<td>1/84</td>
<td>1.19 (0.00–4.00)</td>
<td>9.09</td>
</tr>
<tr>
<td>Chan et al. [10]</td>
<td>1/12020</td>
<td>0.008 (0.00021–0.406)</td>
<td>9.09</td>
</tr>
<tr>
<td>Li et al. [15]</td>
<td>0/1060</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Liu et al. [16]</td>
<td>1/197</td>
<td>2.03 (0.56–5.12)</td>
<td>0.51</td>
</tr>
<tr>
<td>Patients &lt; 14 years</td>
<td>3/156</td>
<td>1.92 (0.40–5.52)</td>
<td>0.43</td>
</tr>
<tr>
<td>Primary school children</td>
<td>1/453</td>
<td>0.22 (0.01–1.22)</td>
<td>5.04</td>
</tr>
<tr>
<td>Patients &gt; 18 years</td>
<td>1/502</td>
<td>0.20 (0.01–1.10)</td>
<td>5.49</td>
</tr>
<tr>
<td>Blood donors</td>
<td>1/875</td>
<td>0.11 (0.0029–0.64)</td>
<td>7.48</td>
</tr>
<tr>
<td>Seto et al. [17]</td>
<td>3/400</td>
<td>0.75 (0.15–2.18)</td>
<td>2.23</td>
</tr>
<tr>
<td>Woo et al. [5]</td>
<td>0/264</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>2003 blood donors</td>
<td>0/264</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Paediatric patients</td>
<td>1/131</td>
<td>0.76 (0.02–4.18)</td>
<td>0.56</td>
</tr>
<tr>
<td>Adult patients</td>
<td>1/131</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Subtotal</td>
<td>0.16</td>
<td>0.00 (0.00–0.17)</td>
<td>47.83</td>
</tr>
<tr>
<td>Health adults pre-2003 epidemic</td>
<td></td>
<td></td>
<td>47.83</td>
</tr>
<tr>
<td>Woo et al. [5]</td>
<td>0/149</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>2000 blood donors</td>
<td>0/149</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Zheng et al. [21]</td>
<td>17/938</td>
<td>1.81 (1.06–2.89)</td>
<td>2.19</td>
</tr>
<tr>
<td>Subtotal</td>
<td>0.54</td>
<td>0.00 (0.00–0.17)</td>
<td>7.40</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>0.29 (0.15–0.44)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Fig. 2. Forest plots of seroprevalence estimates stratified by a priori risk of infection under a random-effects model. The seroprevalence for individual studies is shown as solid squares scaled according to weighting by using the inverse variance method. Error bars indicate 95% CIs. The combined seroprevalence estimates are shown as diamonds that span the 95% CI (* truncated at zero).
Serial confirmatory test protocols against human SARS-CoV

- Chan et al. [10]: 1/12020
- Chow et al. [11]: 0/84
- Gold et al. [12]: 8/767
- Ho et al. [13]: 0/394
- Leang et al. [4]: 2/1068
- Li et al. [15]: 0/1060
- Woo et al. [5]: 2000 blood donors 0/149, 2003 blood donors 3/400, Paediatric patients 1/131, Adult patients 0/264, Health-care workers 0/33, Yu et al. [20]: 0/574

Seroprevalence in %

- Seroprevalence in %: 0.0083 (0.00021–0.046) 14.56
- 0.0083 (0–0.350) 1.14
- 1.04 (0.45–2.04) 1.19
- 0.19 (0–0.98) 7.67
- 0.0083 (0–0.02–0.67) 5.93
- 0.0 (0–0.28) 13.63

Single test protocols or testing against both human and animal SARS-CoV

- CDC [8]: Hospital workers 4/137, Public health officials 1/63, Adults from a clinic 1/84, Chan et al. [9]: 0/674, Li et al. [14]: 0/103, Liu et al. [16]: Patients < 14 years 4/197, Primary school children 3/156, Patients ≥ 18 years 1/453, Blood donors 1/502, Seto et al. [17]: 1/875, Wang et al. [18]: 29/1127, Zheng et al. [21]: 17/938

Seroprevalence in %

- 2.92 (0.80–7.31) 0.08
- 1.59 (0.04–8.53) 0.07
- 1.19 (0.03–6.46) 0.12
- 0.19 (0–0.44) 12.36
- 0.0 (0–2.87) 1.64
- 2.03 (0.56–5.12) 0.17
- 1.92 (0.40–5.52) 0.14
- 0.22 (0.01–1.22) 2.88
- 0.20 (0.01–1.10) 3.39
- 0.11 (0.0029–0.64) 10.67
- 2.57 (1.73–3.67) 0.74
- 1.81 (1.06–2.89) 0.86
- 0.20 (0–0.69) 33.14

Subtotal: 0.05 (0–0.15) 66.86

Total: 0.10 (0–0.20–0.18) 100.00

Fig. 3. Forest plots of seroprevalence estimates (excluding wild animal handlers) stratified by laboratory test strategies under a random-effects model. The seroprevalence for individual studies is shown as solid squares scaled according to weighting by using the inverse variance method. Error bars indicate 95% CIs. The combined seroprevalence estimates are shown as diamonds that span the 95% CI (* truncated at zero).
In addition, there remains much uncertainty as to why two surveys by Woo and colleagues [5] and Gold et al. [12] produced much higher seroprevalence estimates than other studies that also adopted a serial confirmatory testing procedure. We believe that the difference can still be due to false positivity on laboratory testing given the test kit validation procedure adopted by Woo et al. [5] although there is insufficient detail in Gold et al.’s abstract to further appraise the laboratory analysis. In evaluating ELISA for the detection of antibody to nucleocapsid protein, Woo et al. reported a sensitivity of 94.3% and a specificity of 95.3% for IgG antibody by testing specimens from 149 healthy blood donors and 106 SARS patients. Based on subjecting the seven samples out of 149 which gave positive ELISA results to Western-blot testing, they concluded that the specificity of the IgG antibody test was 100%. No other description or information, however, was provided on how the Western-blot assay was evaluated. A larger size of samples from another appropriate source, including potentially interfering samples, would be necessary to confirm the specificity of diagnostic assays. Even with a serial testing algorithm, a small change in specificity may affect the positive predictive value to a great extent, especially when the prevalence of infection here is so low.

Pitfalls, caveats and lessons learned

The first lesson to be drawn concerns sampling methods. With the exception of two studies [4, 10], none of the other serosurveys fully specified the sampling frame, recruitment strategy or response rate. There was also scant attention paid to examining the representativeness of sampled subjects [30].

The second lesson concerns the issue of survival in health-care workers and close contacts. Both groups were clearly exposed, whether protected or not, to a significant infectious source, either through direct contact with SARS patients for whom they cared or with whom they lived in the same household, or via a common environmental point source such as the sewage pipes and bathroom ventilation system in the case of Amoy Gardens in Hong Kong [31]. The fact that they remained asymptomatic or uninfected perhaps implies a systematically different host biology to those who fell sick with the disease. Whether such potential human leukocyte antigen (HLA) allelic differences, that have been suggested to be differentially associated with the clinical severity of SARS, also extend to susceptibility and more specifically to asymptomatic infection has yet been resolved [32]. It would not be surprising if this were the case given previous experience where HLA variations were associated with susceptibility or resistance to malaria, tuberculosis, leprosy, HIV, hepatitis virus persistence and human coronavirus OC-43 infection [33–35]. In this case, the seroprevalence estimates as reported would have been biased either upwards or downwards depending on the effect of the particular HLA polymorphisms.

The third lesson concerns laboratory methods including the use of serial testing procedures and confirmation of screening test results. Our findings indicate that seroprevalence estimates tended to be considerably higher in studies that used only single test protocols compared to those that applied a series of confirmatory tests subsequent to a positive screen. Specificities for the ELISA test against SARS-CoV have been reported to be between 94.3% and 98.5% [4, 36, 37] whereas sensitivities achieved 100% at least 1 month since the acute onset of illness [4, 27]. Given the very low absolute levels of seroprevalence to be detected (i.e. <3% as in the Table), a false-positive ratio of between 1.5% and 5.7% introduces an unacceptably high level of uncertainty in the point estimate.

A related fourth lesson concerns the potential for cross-reactivity between SARS-CoV and other coronaviruses, including the four known human coronaviruses, i.e. OC-229E and OC-43 that cause the common cold and the newly discovered NL63 [38] and HKU1 [39]. NL63 is a group 1 human coronavirus and has been isolated from children and adults with respiratory tract infections as well as immunocompromised adults [40], leading some to propose
that it is a ‘global and seasonal pathogen of both children and adults associated with severe lower respiratory tract illness’ [41]. HCoV-HKU1 is a novel group 2 coronavirus associated with pneumonia recently isolated from two cases in Hong Kong, although its population prevalence has yet to be documented. While this issue remains a potential consideration and various reports have thus far failed to provide conclusive empirical evidence either for [42–44] or against [37] the idea, it is nonetheless important when considering the extent of seroconversion in the population and the laboratory methods employed to reduce this potential bias to a minimum. SARS-CoV is neither a host-range mutant of a known coronavirus nor a recombinant between known coronaviruses but a distinct virus probably with a distant common ancestor to the group 2 bovine and murine coronaviruses [45, 46]. The close similarity between the SARS-CoV open reading frame (ORF) 1b and other coronaviruses [47, 48] as well as the fact that the nucleocapsid or N protein shares common antigenic epitopes with that of antigenic group 1 animal coronaviruses [43] reinforces the need for a cautionary approach in interpreting laboratory results [49–51]. In further support of this, 22 samples out of 33 ELISA-screen positives in the study by Woo et al. [5] reacted against the N protein but turned out to be negative when tested against the spike (S) protein [37]. Indeed, Woo and co-workers [44] recently showed that four out of 31 HCoV-OC43 and OC-229E samples cross-reacted on SARS-CoV ELISA testing. Although none of these four samples were found to contain a specific antibody in the recombinant SARS-CoV spike polypeptide-based Western blot assay, the gold standard to avoid this potential pitfall of cross-reactivity in SARS-CoV antibody detection probably remains the neutralization assay as the final confirmation in the serial testing protocol.

Our findings support the global consensus, from previously available clinical data, that there were very few, if any, confirmed cases of transmission from asymptomatic individuals. It remains possible, although unlikely given the results of published studies reviewed here [4, 10, 15, 16], that the pattern of infection is different in some children and adolescents. Nevertheless, results from an as yet unpublished serosurvey in children from the Amoy Gardens cluster (the largest superspreading event in Hong Kong) may yield new information on this issue.

For population-based studies of communicable disease transmission to provide valid and reliable results we have demonstrated that it is essential that standardized protocols and methods are used to obtain and investigate the sampled subjects. Such an approach would lead to a more rapid development of the evidence base needed to inform public health decision-making in communicable disease control.

ACKNOWLEDGMENTS

We thank Marie Chi for expert secretarial assistance in the preparation of the manuscript. This work was supported in part by the Research Fund for the Control of Infectious Diseases of the Health, Welfare and Food Bureau of the Hong Kong SAR Government and by the European Union. G.M.L. thanks the Takemi Program at the Harvard School of Public Health for hosting his sabbatical leave during which time the revision of this paper was completed. R.M.A. thanks the Wellcome Trust for grant support; N.M.F. and A.C.G. thank the Royal Society for fellowship support.

REFERENCES


