Evaluation of Inapparent Nosocomial Severe Acute Respiratory Syndrome Coronavirus Infection in Vietnam by Use of Highly Specific Recombinant Truncated Nucleocapsid Protein-Based Enzyme-Linked Immunosorbent Assay

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Severe acute respiratory syndrome (SARS) is a recently emerged human disease associated with pneumonia. Inapparent infection with SARS coronavirus (CoV) is not well characterized. To develop a safe, simple, and reliable screening method for SARS diagnosis and epidemiological study, two recombinant SARS-CoV nucleocapsid proteins (N' protein and NΔ21 protein) were expressed in Escherichia coli, purified by affinity chromatography, and used as antigens for indirect, immunoglobulin G enzyme-linked immunosorbent assays (ELISA). Serum samples collected from healthy volunteers and SARS patients in Vietnam were used to evaluate the newly developed methods. The N' protein-based ELISA showed a highly nonspecific reaction. The NΔ21 protein-based ELISA, with a nonspecific reaction drastically reduced compared to that of the nearly-whole-length N' protein-based ELISA, resulted in higher rates of positive reactions, higher titers, and earlier detection than the SARS-CoV-infected cell lysate-based ELISA. These results indicate that our newly developed SARS-CoV NΔ21 protein-based ELISA is not only safe but also more specific and more sensitive method to diagnose SARS-CoV infection and hence a useful tool for large-scale epidemiological studies. To identify inapparent SARS-CoV infections, serum samples collected from health care workers (HCWs) in Vietnam were screened by the NΔ21 protein-based ELISA, and positive samples were confirmed by a virus neutralization test. Four out of 149 HCWs were identified to have inapparent SARS-CoV infection in Vietnam, indicating that subclinical SARS-CoV infection in Vietnam is rare but does exist.

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of SARS infections among laboratory researchers in Singapore, Taiwan, and Beijing, China, has caused great concern about laboratory safety (14, 27, 28). Hence, safer and more convenient methods for SARS diagnosis and large-scale epidemiological studies are needed.

The SARS outbreak in Vietnam began with the admission of a traveler from Hong Kong to the French Hospital, a 56-bed, three-story, privately owned, expatriate-operated hospital located in Hanoi, Vietnam, on 26 February 2003. Within 2 weeks, extensive nosocomial transmission of SARS occurred, mainly among the hospital staff. On 12 March, this hospital was closed to new admissions, with the exception of hospital workers. On 28 April 2003, Vietnam became the first country to recognize and contain a SARS outbreak, as announced by the WHO. In Vietnam, SARS occurred mainly in the above-mentioned French Hospital, among HCWs, and the existence of a clear index case transmitting the virus to close contacts was identified. All of these characteristics made this place ideal to study the epidemiology of this disease.

To undertake the study of SARS-CoV subclinical infection among Vietnamese HCWs, we developed a truncated SARS-CoV N protein-based ELISA system by using recombinant techniques. This new ELISA system is safer, more specific, and more sensitive for the diagnosis of SARS-CoV infection. The present study describes the existence of asymptomatic SARS-CoV infection in Vietnam, as proven by use of this new screening method and confirmed by a virus neutralization test. Data on the sensitivity and specificity of this SARS-CoV ELISA system based on recombinant truncated N proteins are discussed.

MATERIALS AND METHODS

Serum samples. One hundred seventy-five serum samples from healthy volunteers from Hanoi, collected before the SARS outbreak, were used as negative controls. Serum samples from 149 HCWs who were in close contact with SARS patients at the French Hospital in Hanoi were included in this study. Among them, 37 were probable SARS cases, per the WHO case definition, and 112 were symptom free. Serum samples from those 37 probable SARS cases and 112 symptom-free HCWs were collected from 11 March to 3 April 2003.

RNA extraction. SARS-CoV strain Hanoi 01-03, isolated from a Vietnamese patient, was propagated in a Vero E6 cell line maintained at 37°C in Eagle's minimum essential medium supplemented with 2% fetal calf serum and 0.2 mM of each nonessential amino acid for 4 days. Upon observation of 80 to 100% cytopathic effect (CPE), the infected culture supernatant was clarified by light centrifugation at 2,000 × g for 10 min. Viral RNA was extracted from 140 μl of infected culture supernatant by using the QIAamp viral RNA minikit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The extracted RNA was eluted in 60 μl of elution buffer and then used as the template for reverse transcription (RT-PCR).

Construction of recombinant plasmids. The gene for the SARS-CoV N protein was amplified by RT-PCR as previously described (9). PCR amplification was carried out using primers 5'-TCTAGATGGCCATCAATACCAAA-3' and 5'-TGACTGATCATAGAATTTTATA-3' to generate a gene for the N protein (the N protein with the four leading amino acids [aa] clipped) and primers 5'-AGAAGGATCCCTTCCCTACGGCGCT-3' and 5'-TGTTTAT-3' to generate a gene for the N121 protein (a second N protein construct with 121 aa of the N terminus truncated). The sense and reverse primers contained BamHI and Sal restriction sites (underlined), respectively. The 1.3-kb and 0.9-kb PCR-amplified DNA fragments were digested with BamHI and Sal and subsequently cloned into the corresponding restriction site of the pQE30 vector (QIAGEN, Hilden, Germany). Two expression products, one encompassing aa 5 to 422 and another encompassing aa 122 to 422 of the SARS-CoV N protein, were obtained, and both constructs contained a vector-derived His tag (histidine hexamer) at their N termini. The resultant recombinant proteins were designated SARS N' and SARS N121, respectively.

Expression and purification of the recombinant SARS-CoV N proteins. The recombinant SARS-CoV N proteins were expressed by inserting the recombinant plasmids containing the SARS-CoV N' and SARS-CoV N121 sequences, inserted Escherichia coli strain XL1-Blue and then cultured at 30°C in Luria-Bertani medium containing 100 μg/ml of ampicillin. When the optical density at 600 nm (OD600) of the culture reached 0.5, expression of the recombinant proteins was induced by the addition of 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3 h. The cells were harvested by centrifugation, washed in phosphate-buffered saline (PBS) solution, resuspended in 10 mM PBS (pH 7.5)-500 mM NaCl, and frozen at −80°C. After being frozen and thawed three times, the cell suspension was sonicated for 2 min with an interval of 1 s between pulses and centrifuged at 30,000 × g for 15 min at 4°C. The supernatant was then applied to a Talon IMAC resin column (Clontech). After being washed with 10 mM PBS-500 mM NaCl containing 20 mM imidazole, the purified proteins were then eluted with 10 mM PBS (pH 7.5)-500 mM NaCl containing 250 mM imidazole. The protein solutions were aliquoted and stored in a final concentration of 10% glycerol at −80°C until use. Protein concentrations were determined by the Bradford method (1a) with a protein assay reagent kit (Bio-Rad), and the purity of the proteins was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Western blot analysis. Western blotting was performed as described by Towbin et al. (21). Briefly, proteins separated in a 10% polyacrylamide gel were transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilon; Millipore) by using a semidy electrophoresis blotter (Sartorius, Germany). The membrane was initially blocked with Blockace (Yukijirushi, Sapporo, Japan) overnight at 4°C; subjected to reaction with mouse antihistidine serum (1:200 dilution; Amsterdam Biosciences, NJ); SARS-CoV-immunized rabbit serum (1:200 dilution; supplied by the National Institute of Infectious Disease, Japan); or SARS patient serum (1:100 dilution) for 1 h at 37°C; and then incubated with rabbit anti-mouse immunoglobulin G (IgG) conjugated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG-peroxidase conjugate or goat anti-human IgG-peroxidase conjugate (1:1,000 dilution) (all conjugates were procured from American Qualex, California) for 1 h at 37°C. Finally, the reaction results were visualized by dimethylamino benzidine (DAB) staining.

ELISA using the recombinant nucleocapsid proteins. A total of 175 serum samples collected from healthy volunteers in Vietnam before the SARS outbreak and 150 serial serum samples collected from 37 patients with pneumonia were used for the assessment of the IgG antibody ELISA. The optimal concentrations of recombinant N' and N121 proteins were determined by checkerboard titration with different dilutions of coating recombinant proteins. The optimal amount of antigen for plate coating was 0.13 μg per ELISA well for each recombinant protein. Ninety-six-well Nunc immunoplates (Roskilde, Denmark) were coated with recombinant N' or N121 protein antigens in carbonate buffer (pH 9.6) overnight at 4°C and then blocked with Blockace for 1 h at room temperature. After the immunoplates were washed six times with PBS-Tween 20, 100 μl of 1:100 human serum diluted in Blockace was added to each well and incubated for 1 h at 37°C. Then, after the plates were washed six times with PBS-Tween 20, 100 μl of 1:3,000-diluted horseradish peroxidase-conjugated goat anti-human IgG (American Qualex, California) was added to each well, and the plates were incubated at 37°C for 1 h. After six more washes with PBS-Tween 20, 100 μl of diluted o-phenylenediamine was added to each well and incubated in the dark at room temperature for 10 min. The reaction was then stopped by adding 100 μl of 1 N H2SO4 to each well. OD492 for each well was measured with a 620-nm reference filter. Each sample was tested in duplicate, and the mean OD for each sample was calculated. ELISA titers were calculated from standardized reciprocal dilution values by using Thermo-Labsystem's Ascent photospectrometric data analysis software, version 2.6. Each serum sample was checked twice, and the mean antibody titer were recorded.

ELISA using SARS-CoV-infected cell lysates. An ELISA using SARS-CoV-infected Vero E6 cell lysates was performed according to the protocol of the Centers for Disease Control and Prevention, Atlanta, Georgia (10). The cell lysates of the SARS-CoV-infected and -uninfected Vero E6 cells were supplied by the Centers for Disease Control and Prevention.

Virus neutralization test. A virus neutralization test was done under biosafety level 3 conditions by using the 50% tissue culture infective dose method. Briefly, sera were heat inactivated at 56°C for 30 min and then serially diluted twofold from 1:10 to 1:1,280. An equal volume of virus-infected cell culture fluid with a multiplicity of infection (MOI) of 0.1 was added to each well. After 1 h at 37°C, each well was washed twice with PBS-Tween 20. Finally, the reaction results were visualized by dimethylamino benzidine (DAB) staining.
RESULTS

Expression and purification of recombinant SARS-CoV N proteins. The recombinant SARS-CoV N proteins, encompassing amino acid residues 5 to 422 and 122 to 422 of the nucleocapsid protein, were amplified by RT-PCR and cloned into the BamHI and SalI sites of the expression vector pQE30 in frame and downstream of the six-histidine tag. The recombinant proteins were successfully expressed in *E. coli* and purified by use of a Talon metal affinity column under natural conditions. Analysis of purified recombinant proteins by SDS-PAGE and Coomassie blue staining revealed, as predicted, single protein bands of 46 kDa and 32 kDa for the two recombinant SARS-CoV N/H11032 and N/H9004121 proteins, respectively (Fig. 1).

The identities of the recombinant SARS-CoV N/H11032 and N/H9004121 proteins were further confirmed by Western blot assay with mouse antihistidine serum, SARS-CoV-immunized rabbit serum, and SARS patient serum (Fig. 2).

Calibration of ELISA for recombinant N/H11541 and N/H9004121 proteins. In order to determine the basal titers and cutoff values, serum samples collected from 175 healthy volunteers in Vietnam before the SARS outbreak were used for the assessment of the indirect IgG ELISA for recombinant SARS-CoV N/H11032 and N/H9004121 proteins. When the N/H11032 protein was used as the coating antigen, 38 out of the 175 serum samples showed titers higher than 100, ranging from 100 to 3,200. On the contrary, when the SARS-CoV N/H9004121 protein was used, only 11 out of 175 samples showed titers between 100 and 260. Further analysis of all of these positive samples by Western blotting also confirmed the reactivities (data not shown), indicating that the positive reaction by ELISA was not due to the potential interaction between residual *E. coli* antigens and naturally occurring antibodies against *E. coli* in human sera. We chose seven serum samples which showed high titers by the N/H11032 protein-based ELISA but were negative by the N/H9004121 protein-based ELISA and confirmed their reactivities by Western blot assay. As shown in Fig. 3, these seven samples were positive by the N/H11032 protein-based Western blot assay but negative by the N/H9004121 protein-based Western blot assay. This indicates...
that the cross-reactivity observed for these healthy volunteer donors was caused by the N terminus of the SARS-CoV N protein, which has conserved motifs with other coronaviruses (18).

**Evaluation of recombinant NΔ121 protein-based ELISA.** Serial serum samples collected from 37 probable SARS cases were assessed by the recombinant NΔ121 protein-based ELISA to determine the presence of IgG antibodies to the SARS-CoV N protein. Thirty-six out of 37 patients (97.3%) showed specific IgG seroconversion, with titers ranging from 600 to 204,800. All positive samples were confirmed by Western blot assay. Among those 36 IgG-positive patients, anti-NΔ121 protein IgG seroconversion rates were 22.2%, 69.4%, and 100% within 7 days, 2 weeks, and 3 weeks after the onset of fever, respectively. One patient did not have detectable IgG antibodies to the NΔ121 protein. This patient’s serum samples were collected only up to day 14 after the onset of disease; hence, a late seroconversion to SARS-CoV positivity might be the explanation for the lack of reaction. We also tested these patients’ serum samples with the recombinant NΔ121 protein-based ELISA and found that the reaction range and titers showed no difference from those obtained by the NΔ121 protein-based ELISA (data not shown).

In the recombinant NΔ121 protein-based IgG ELISA, the highest IgG titer observed for the group of 175 healthy volunteers was 260, and the lowest IgG titer observed for the SARS patients was 600. Therefore, 300 was set as the cutoff titer for our NΔ121 protein-based IgG ELISA. After the cutoff titer was set at 300, the clinical specificity of the NΔ121 protein-based IgG ELISA was 100%.

**Comparison of recombinant NΔ121 protein-based ELISA with SARS-CoV-infected cell lysate-based ELISA.** We compared the recombinant NΔ121 protein-based ELISA with the SARS-CoV-infected cell lysate-based ELISA by using serial serum samples collected from 37 probable SARS cases. As shown in Table 1, the recombinant NΔ121 protein-based ELISA showed a seroconversion rate of 97.3%, while that of the SARS-CoV-infected cell lysate-based ELISA was only 56.8%. The highest titers detected were 204,800 and 6,400, and the earliest seroconversion times detected after the onset of illness were 6 days and 9 days for the NΔ121 protein-based ELISA and the SARS-CoV-infected cell lysate-based ELISA, respectively. For the 21 samples that were positive by both methods, the NΔ121 protein-based ELISA detected seroconversion earlier than the SARS-CoV-infected cell lysate-based ELISA in 16 samples and on the same day for the other 5 samples. The median seroconversion times were 12 days and 19 days, and the geometric mean titers were 11,768 and 2,540 for the NΔ121 protein-based ELISA and the SARS-CoV-infected cell lysate-based ELISA, respectively.

**Identification of inapparent SARS infection.** Using the recombinant NΔ121 protein-based IgG ELISA, we screened serum samples collected from 112 symptom-free Vietnamese HCWs who had close contact with SARS patients. Four of those 112 HCWs had anti-SARS-CoV NΔ121 protein antibodies. Three of them, for whom paired samples were tested, showed IgG seroconversion, and the fourth one showed a titer of 8,400 for a single blood serum sample. All four cases were confirmed by Western blotting and neutralization tests. The neutralizing antibody titers observed for these four HCWs were 24, 48, and 114, respectively. For the three individuals for whom paired serum samples were available, the first serum sample was negative by neutralization test. This clearly demonstrated that these four HCWs acquired inapparent SARS-CoV infections during the SARS outbreak. As shown in Table 2, out of 149 HCWs who were in close contact with SARS patients at the French Hospital, 37 (24.8%) had SARS and 4 (2.7%) experienced asymptomatic infection (P < 0.001), indicating the existence of a relatively low rate of inapparent SARS-CoV infection among HCWs in Vietnam.

**DISCUSSION**

There are several reports on the use of recombinant SARS-CoV nucleocapsid protein for the serodiagnosis of SARS-CoV clinical infection, indicating that SARS-CoV nucleocapsid protein is highly immunogenic and abundantly expressed during infection and is therefore useful for the diagnosis of SARS-CoV infection (5, 7, 20). Since the N proteins of known coronaviruses are relatively conserved, it is important to ascertain whether SARS-CoV N protein is cross-reactive with other coronaviruses especially with antisera of other human coronaviruses. Sun and Meng reported that the N protein of coronaviruses react strongly with polyclonal antisera of known coronaviruses and especially with antisera of other human coronaviruses. Therefore it is important to ascertain whether SARS-CoV N protein is cross-reactive with other coronaviruses especially with antisera of other human coronaviruses.

**TABLE 1. Comparison of recombinant-NΔ121 protein-based ELISA and SARS-CoV-infected cell lysate-based ELISA**

<table>
<thead>
<tr>
<th>Method</th>
<th>No. (% of patients)</th>
<th>Antibody titer of positive samples</th>
<th>Day of detection of seroconversion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG negative</td>
<td>IgG positive</td>
<td>Approx range</td>
</tr>
<tr>
<td>NΔ121-based ELISA</td>
<td>1</td>
<td>36 (97.3)</td>
<td>600–204,800</td>
</tr>
<tr>
<td>SARS-CoV-infected cell lysate-based ELISA</td>
<td>16</td>
<td>21 (56.8)</td>
<td>400–6,400</td>
</tr>
</tbody>
</table>

a Sera from 37 SARS patients were used.  
b Positive rate at 3 weeks after onset of fever.  
c For the 21 patient serum samples which were positive for IgG by both methods.  
d For the 21 patient serum samples which were positive for IgG by both methods.

**TABLE 2. Rates of clinical and inapparent SARS-CoV infection in HCWs in Vietnam**

<table>
<thead>
<tr>
<th>Symptom status</th>
<th>No. of HCWs positive for SARS/total no of HCWs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS patient</td>
<td>37/149 (24.8)</td>
</tr>
<tr>
<td>Subclinical SARS infection</td>
<td>4/149 (2.7)</td>
</tr>
</tbody>
</table>

a A P value of <0.001 was determined by the χ² test.
animal coronaviruses belonging to antigenic group I (19). Woo et al. also reported that recombinant SARS-CoV nucleocapsid protein-based ELISA and Western blot assays have high rates of false positivity (24). In a recent report, Woo et al. found that 3 out of 21 and 1 out of 7 convalescent-phase serum samples from persons infected with human coronavirus (HCoV)-OC43 and HCoV-229E, respectively, tested positive by recombinant SARS-CoV nucleocapsid protein-based ELISA, suggesting that there is cross-reactivity between SARS-CoV N protein and HCoV-OC43 and HCoV-229E N proteins (25). It has been reported that antibodies against human 229E- and OC43-like coronaviruses are widespread within the human population (1, 16). Our recombinant SARS-CoV N’ protein-based ELISA revealed that 38 out of 175 serum samples from healthy volunteers collected before the SARS outbreak had titers higher than 100, ranging from 100 to 3,200. Taken together, these facts indicate that the reactivity we observed for the healthy volunteer sera presented in this study was probably due to cross-reactivity with existing antibodies to other circulating coronaviruses. This clearly indicates that care should be taken while interpreting assay results when the full-length recombinant N protein of SARS-CoV, whole-virus antigen extracts, or virus-infected cells are used as reagents for the diagnosis of SARS-CoV infections in humans and animal species.

The predicted N protein of SARS-CoV is a highly charged basic protein of 422 amino acids with seven successive hydrophobic residues near the middle of the protein. Although the overall amino acid sequence homology of the SARS-CoV N protein to other human coronaviruses is very low (32.7% between SARS-CoV and human coronavirus OC43 and 21.3% between SARS-CoV and human coronavirus 229E), a highly conserved motif (FYYLGTGP) occurs in the N-terminal half of all coronavirus N proteins, and other conserved residues are reported to occur near this highly conserved motif (18). Using synthetic peptides, Wang et al. found that the most immunoreactive epitopic site in the SARS coronavirus N protein is located at its COOH terminus (23). In theory, these findings make it possible to remove the N terminus of the SARS-CoV N protein, which contains the conserved motif of all coronavirus N proteins, to reduce cross-reactivity in SARS diagnosis. We expressed a truncated SARS-CoV NΔ121 protein, in which the highly conserved motif (FYYLGTGP) was deleted. When this NΔ121 protein was used as the coating antigen for our ELISA, 11 out of 175 samples showed weak reactions, with titers ranging from 100 to 260, much fewer positive samples with titers much lower than those observed when the N’ protein was used. This was confirmed by Western blot analysis (Fig. 3). Our results further supported the fact that the SARS-CoV N protein shows cross-reactivity with those of other coronaviruses and that such cross-reaction is caused by the conserved motif present within the N terminus of the protein. The fact that 11 samples also showed, although with low titers, reactivity against the SARS-CoV NΔ121 protein suggests that some minor common motifs might still exist within the truncated NΔ121 protein. Finally, to solve cross-reactivity problems, 300 was set as the cutoff titer, and all of these weak reactions were interpreted as negative in our ELISA system.

To assess the sensitivity of the NΔ121 protein-based IgG ELISA, serially collected serum samples from 37 probable SARS cases were examined. Thirty-six patients (97.3%) showed IgG seroconversion, and the serum titers ranged from 600 to 204,800. Among these 36 positive cases, the anti-NΔ121 protein IgG seroconversion rates after the onset of illness were 22.2% by the first week, 69.4% by the second week, and 100% by the third week. The recombinant NΔ121 protein-based ELISA was more sensitive and could detect seroconversion earlier than the virus-infected cell lysate-based ELISA system (Table 1). Our newly developed SARS-CoV NΔ121 protein-based IgG ELISA is a safe, specific, and sensitive test for the diagnosis of SARS-CoV infection. Therefore, it may be useful for clinical diagnosis and large-scale seroepidemiological studies.

Using the SARS-CoV NΔ121 protein-based IgG ELISA, we screened serum samples from 112 symptom-free HCWs who had direct contact with SARS patients in Vietnam. We found that four symptom-free HCWs had developed antibody against SARS-CoV. In addition, a positive reaction was confirmed by Western blotting and virus neutralization tests. This proved that all four symptom-free HCWs truly had subclinical SARS-CoV infections. The comparison of rates of symptomatic and asymptomatic SARS-CoV infections among HCWs in Vietnam revealed that symptomatic infection was more common than subclinical infection (Table 2). Therefore, our data strongly indicate that subclinical SARS-CoV infection actually does exist, although at quite a low rate.

Vietnam was the first country in which SARS was acknowledged. At the beginning of the outbreak, the severity of the disease was not recognized; hence, no effective prevention measures were taken. Therefore, within 2 weeks, extensive nosocomial transmission occurred among the hospital staff at the French Hospital. Contact-tracing data revealed that all of the four symptom-free HCWs from this study who had antibodies against SARS-CoV were in direct contact with the index case; however, they did not develop SARS but experienced only subclinical infection, as proven by seroconversion. There have been several reports dealing with subclinical or atypical forms of SARS. Lee et al. reported a case of possible asymptomatic SARS-CoV infection in Hong Kong (11). Li et al. reported that, among 125 people exposed to SARS patients, 20 developed SARS and 2 had nonpneumonic infections (13). In addition, Ho et al. reported that 8 out of 372 HCWs in a teaching hospital in Singapore were positive for SARS-CoV antibody. Among them, six were probable or suspected SARS cases; however, two had only fever, indicating that a small number of mildly symptomatic individuals existed, although no asymptomatic individuals were found (6). Chow et al. reported that with a dot blot ELISA combined with a virus neutralization test of 87 HCW serum samples, no subclinical SARS was found (4). In addition, Chan et al., using an indirect immunofluorescence assay, examined 674 HCWs from a hospital in which a SARS outbreak had occurred, and none of the HCWs had antibodies to SARS-CoV (3). In a later report, Ip et al. found that 3 out of 131 HCWs who worked in SARS medical wards had subclinical infections (8). All of these studies used inactivated virus-infected cell extract-based ELISA or virus-infected cell-based immunofluorescence assays for diagnosis; however, as shown in our study, the inactivated virus-infected cell extract-based ELISA detected SARS antibodies with low sensitivity. Besides, with these methods, the possibility of cross-reaction with other human coronaviruses exists. On the con-
trary, our detection system, which consists of a truncated N protein-based ELISA for the screening of symptom-free HCWs and confirmation by a virus neutralization test, is more sensitive and more specific, giving much more reliable results to elucidate subclinical SARS-CoV infection.

On the other hand, Woo and colleagues, using a recombinant N protein-based ELISA combined with Western blot analysis for the detection of SARS-CoV N and S proteins, found that 3 out of 400 healthy blood donors and 1 out of 131 nonpneumonic inpatients were positive for SARS-CoV antibodies. Therefore, they concluded that subclinical or nonpneumonic SARS-CoV infections are more common than SARS pneumonia (24). As suspected by other researchers, this could be due to cross-reactivity or misinterpretation of their results (29).

Considering all previously reported information and our data, it is clear that subclinical and mild forms of SARS-CoV infection are rare but existent. This may be the reason why control measures, such as quarantining patients, worked effectively to contain the outbreak in Vietnam. SARS-CoV was a newly emerged virus, and humans did not have immunity against it; as a result, most of the people infected developed clinically symptomatic infections. This implies that another outbreak could occur, because only very few people may have acquired immunity through subclinical infections and, hence, the majority of the population might remain susceptible to the disease.

In conclusion, we developed a SARS-CoV N121 protein-based IgG ELISA which is a specific, and sensitive test for the diagnosis of SARS-CoV infection. We also showed that subclinical SARS-CoV infection, though rare, does indeed occur.

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