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Prevalence of non-pneumonic infections with SARS-correlated virus

Sir—Patrick Woo and colleagues (Mar 13, p 841)¹ suggest that non-pneumonic severe acute respiratory syndrome (SARS)-virus infections are more common than SARS-virus pneumonia in Hong Kong on the basis of a serological survey of about 1000 non-pneumonic healthy individuals of different groups and periods. We query the validity of the authors' experimental approach, which involved western blots only, on the confirmation of the ELISA positive samples.

Woo and colleagues used SARS-virus nucleocapsid protein expressed in *Escherichia coli* as the coating antigen in their diagnostic ELISA, and the positive samples were confirmed by two "independent" western blots against *E coli*-expressed nucleocapsid and spike polypeptides. These confirmatory assays were aimed at eliminating the false positives potentially caused by the cross-reactivity between antibodies against the nucleocapsid proteins of other human coronaviruses and those of SARS virus. However, the authors overlooked the possibility of an interaction between residual *E coli* antigens and naturally occurring antibodies against *E coli* in the hosts as another potential source of false positives. They did not discuss the purity of the purified *E coli*-expressed antigens, which is a crucial factor in the interaction because a tiny amount of residual *E coli* antigen is sufficient to interfere with the diagnostic assay. Moreover, the presence of naturally occurring antibodies against *E coli* in serum from healthy individuals has been reported previously.²⁻⁴ Such antibodies are mainly present because of infection with common *E coli* strains and the natural intestinal flora in healthy individuals.

Woo and colleagues' confirmation of the four positive samples by use of two western-blot assays is not persuasive. First, the authors did not present the corresponding western-blot results to show the binding specificity—ie, a single prominent band of the target antigen—which is needed to exclude the possibility of cross-reactivity between the host's antibodies and the residual *E coli* antigens. Second, the molecular size of both target antigens (ie, spike and nucleocapsid) are

virtually the same (about 50 kDa), which could create a problem with respect to the validity of the diagnostic assay if contaminating *E coli* antigens of a similar size are present. In our opinion, Woo and colleagues should use other independent diagnostic systems to control for various sources of false positives—eg, western-blot assays with antigens of significantly different molecular sizes or antigens expressed in another system, ELISA with viral cell-culture extract as the coating antigen, or indirect immunofluorescence.

A similar study was done by the Center for Emerging Infectious Disease of the Chinese University of Hong Kong.⁵ The seroprevalence of asymptomatic, or non-pneumonic, SARS-virus infection in that study was 0.009% (one in 12 000), which is significantly lower than that reported by Woo and colleagues (0.48%). The differences between these two studies are unknown, but bearing in mind their importance with respect to the possible human reservoir for SARS-virus infection in Hong Kong, such serological studies must be carefully designed to eliminate false positives.

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- 1 Woo PC, Lau SK, Tsoi HW, et al. Relative rates of non-pneumonic SARS coronavirus infection and SARS coronavirus pneumonia. *Lancet* 2004; **363**: 841–45.
- 2 Griffiths E, Stevenson P, Thorpe R, et al. Naturally occurring antibodies in human sera that react with the iron-regulated outer membrane proteins of *Escherichia coli*. *Infect Immun* 1985; **47**: 808–13.
- 3 Henriksen AZ, Maeland JA. Serum antibodies to outer membrane proteins of *Escherichia coli* in healthy persons and patients with bacteremia. *J Clin Microbiol* 1987; **25**: 2181–88.
- 4 Sanches MI, Keller R, Hartland EL, et al. Human colostrum and serum contain antibodies reactive to the intimin-binding region of the enteropathogenic *Escherichia coli* translocated intimin receptor. *J Pediatr Gastroenterol Nutr* 2000; **30**: 73–77.
- 5 Chinese University of Hong Kong. Territory-wide SARS seroprevalence study suggests Hong Kong is not a human reservoir for SARS coronavirus. Shatin: Chinese University of Hong Kong, 2004. <http://www.cuhk.edu.hk/ipro/pressrelease/040315e.htm> (accessed Apr 26, 2004).

Sir—Patrick Woo and colleagues¹ use an IgG antibody test combined with western-blot analysis to demonstrate the existence of subclinical or non-pneumonic infections with SARS virus and the prevalence of such cases compared with pneumonic SARS cases. However, the four patients identified as having non-pneumonic SARS were among a group of 33 positive by the authors' ELISA, of whom 26 were also positive by nucleocapsid western blot. Thus, 22 samples turned out to be positive for antibodies against SARS virus nucleocapsid protein, but negative for antibodies against SARS virus spike protein.

This high rate of false-positive samples, as the authors qualified them, is difficult to explain because serum samples from patients with OC43 and 229E human coronavirus infections are not cross-reactive with SARS virus.² Therefore, since SARS-virus structural proteins have shown close sequence identity with those of other viruses, how can the authors be so conclusive with regard to samples that are double-positive by spike and nucleocapsid western blots? Is a second antigen really sufficient to draw such a conclusion, or could it be that spike western blot also led to some false-positive results? The large number of serum samples from non-pneumonic patients that were reactive with SARS virus nucleocapsid protein sheds doubt on the ability of western blotting with SARS virus spike protein to identify truly positive samples, since no clear proof of its specificity over the corresponding blot with nucleocapsid protein has been provided.

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Sir—Patrick Woo and colleagues¹ provide evidence of subclinical or non-pneumonic infections with the virus