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Antibody against nucleocapsid protein predicts susceptibility to human coronavirus infection

Dear Editor,

We read with interest the study that antibodies induced by receptor binding domain in spike protein of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) do not cross-neutralize Middle East respiratory syndrome coronavirus (MERS-CoV) reported in this journal recently.[1]

To date, six CoVs, including human CoV-229E, -NL63, -OC43, -HKU1, SARS-CoV, and MERS-CoV, are known to infect humans. The number of MERS-CoV infection cases in the world has sharply increased since mid-March 2014 and the infections have spread from the Middle East to Europe, North Africa, Asia, and America. The World Health Organization (WHO) has encouraged all countries to monitor MRES-CoV and to carefully review any unusual patterns. However, for mild or unusual symptomatic infection, it is not always possible to identify the infection with MERS-CoV using PCR assay. Hence, it is important to perform seroepidemiological studies in natural populations to analyze HCoVs' epidemiologic spectrum.

The CoV nuleocapide (N) protein is abundantly produced during infection and exhibits strong immunogenicity, which can act as an ideal antigen for viral antibody detection.[2–4] However, the antigenic and serologic relationship between N proteins within subgroups of the six HCoVs, such as NL63 and 229E (both subgroup 1b), OC43 and HKU1 (both subgroup 2a), has not been fully understood, which can affect seropositive data of HCoVs. A recent study showed that BtCoV HKU5 and BtCoV HKU4 N proteins within the 2c subgroups share cross-reactive epitopes with MERS-CoV. In addition, BtCoV

HKU3 and BtCoV 279 N proteins within the 2b subgroups share cross-reactive epitopes with SARS-CoV.[5]

To evaluate the cross-reactivities among HCoVs, we developed a competitive ELISA (cELISA) as previously described for detecting anti-N IgG antibodies of six HCoVs.[6] To this end, we first identify HCoV-positive (including HCoV-229E, -OC43 -NL63, -HKU1, and SARS-CoV) and -negative human serum samples by Western blot analysis. Using these positive controls, we found that 1.0 μg/ml of competing N protein was sufficient for the competition assays in cELISA (Fig. 1A–E).

We then evaluated the possible cross-reactivity among N proteins using cELISA with the positive control sera. Our results suggest that HCoV-229E and -NL63 (subgroup 1b), and HCoV-OC43 and -HKU1 (subgroup 2a) share subgroup cross-reactive epitopes among their N proteins (Fig. 1A–E). However, no cross-reactivity was observed between the N proteins across groups or subgroups. Therefore, cELISA assays were performed with N proteins between HCoV-NL63 and -229E, and HCoV-OC43 and -HKU1 to minimize the cross-reactivity for seroprevalence determination. Since we did not have access to the positive human serum against MERS-CoV, the cross-reactivity of MERS-CoV antibodies with other HCoV N proteins could not be determined. We performed cELISA of MERS-CoV competing with the other five N proteins together.

To evaluate the seroprevalence of HCoVs in China, we determined the cut-off values of the cELISA for six HCoVs as previously described.[7,8] We obtained the cut-off values of HCoV-NL63, -229E, -OC43, -HKU1, SARS-CoV, and MERS-CoV as 0.25, 0.25, 0.23, 0.25, 0.27, and 0.25, respectively. A tested sample was considered positive if its A450 was above the cut-off value.

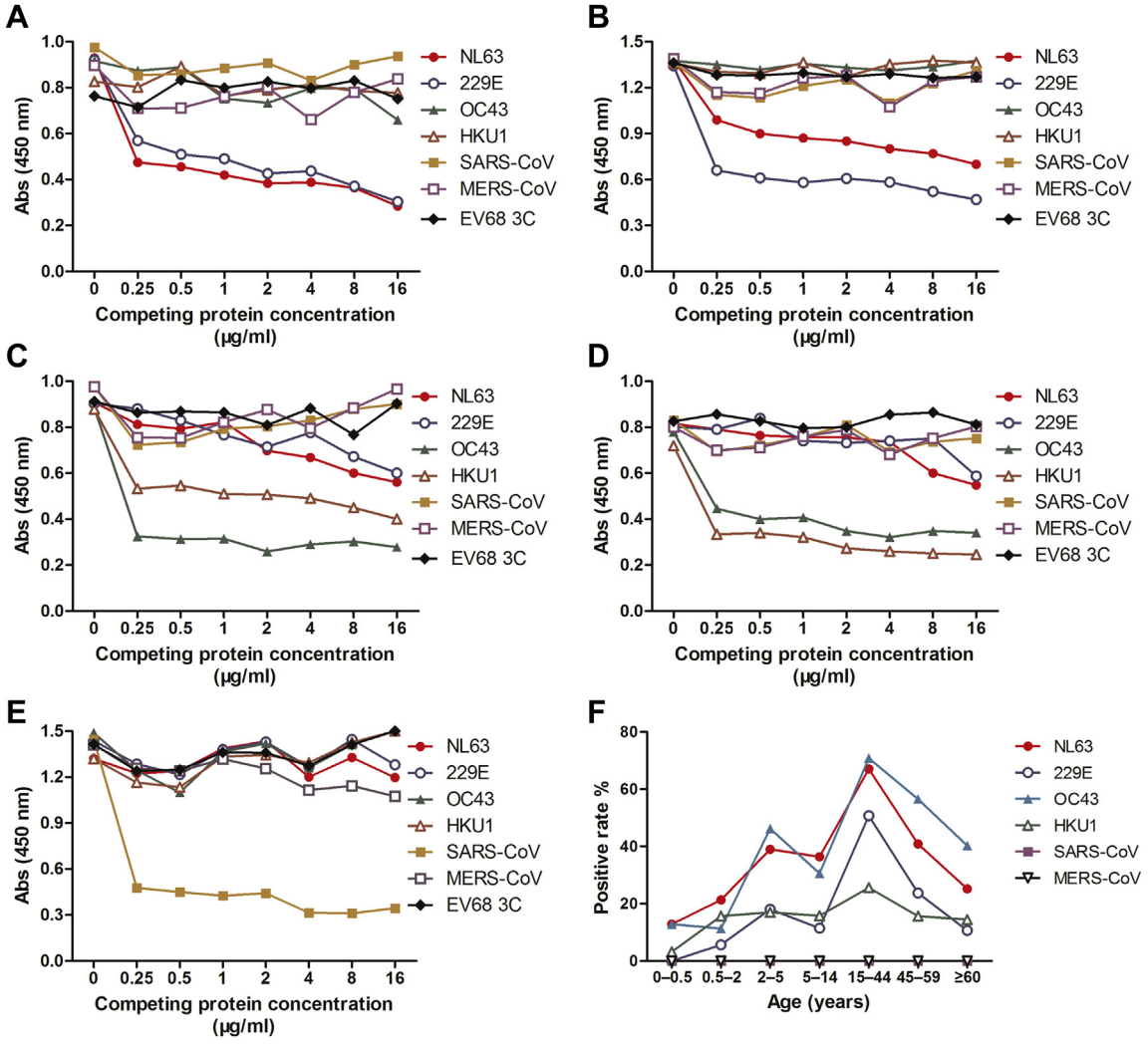

Figure 1 Cross-reactivity among human coronaviruses (HCoVs) and seropositive rates of IgG antibodies against HCoVs in different age groups. Human positive sera against HCoV-NL63 (A), -229E (B), -OC43 (C), -HKU1 (D) and SARS-CoV (E) were tested for reactivity to N proteins of HCoV-NL63, -229E, OC43, -HKU1, SARS-CoV and MERS-CoV, respectively, using a competive ELISA assay. EV68 3C protein was used as the control. The absorbance values (Abs) at 450 nm for each concentration of coating antigens are shown on the y-axis; the competing protein concentrations in ELISA assay are shown on the x-axis. (F) IgG antibodies against HCoV-NL63, -229E, -OC43, -HKU1, SARS-CoV and MERS-CoV were detected by competition ELISA at a dilution of 1:200. All serum samples were grouped based on age, as indicated by the x-axis labels.

Table 1 Comparison of serum IgG antibody levels against N proteins for ARTI patients positive and negative for HCoVs.

HCoVs	Positive			Negative			χ^2, P value
	All	IgG+ (%)	IgG− (%)	All	IgG+ (%)	IgG− (%)	
NL63	21	5[a](23.8)[b]	16 (76.2)	246	142 (57.7)	104 (42.3)	$\chi^2 = 7.68$, P = 0.0056
229E	30	7 (23.3)	23 (76.7)	246	124 (50.4)	122 (49.6)	$\chi^2 = 6.81$, P = 0.00906
OC43	38	7 (18.4)	31 (81.6)	246	123 (50.0)	123 (50.0)	$\chi^2 = 11.98$, P = 0.00054
HKU1	26	6 (23.1)	20 (76.9)	246	133 (54.1)	113 (45.9)	$\chi^2 = 7.84$, P = 0.00511

[a] Number of positive samples.
[b] Percentage of positive samples.

Then we tested anti-N IgG in sera from 695 healthy adults ≥15 yr by cELISA (Fig. 1F). We obtained seroprevalences of 50.8% for HCoV-229E, 67.1% for -NL63, 70.8% for -OC43, and 25.6.7% for -HKU1 for the age group of 15—44 year olds. Seroprevalences decreased with age, with 10.7% for HCoV-229E, 25.2% for -NL63, 40.3% for -OC43, and 14.5% for -HKU1 for old adults of ≥60 yr. No SARS-CoV and MERS-CoV IgG were detected in these serum samples. The seropositive rates of HCoV-NL63 and -OC43 were higher than those of HCoV-229E and -HKU1 (χ^2 tests. $\chi^2 = 130.8$, $P = 3.9 \times 10^{-29}$ for HCoV-NL63 *vs* -229E and -HKU1; $\chi^2 = 239$, $P = 1.2 \times 10^{-52}$ for HCoV-OC43 *vs* -229E and -HKU1) (Fig. 1F). Our data suggest that there is an age-related waning of HCoV-229E, -NL63, -OC43, and -HKU1 specific antibodies.

The seroprevalence of 492 healthy children ≤14 yr were 12.4% for HCoV-229E, 33.7% for -NL63, 32.5% for -OC43, 15.4% for -HKU1, 0% for SARS-CoV, and 0% for MERS-CoV IgG (Fig. 1F). Anti-N-IgG antibodies of HCoV-229E, -NL63, -OC43, and -HKU1 were detected in children between 0.5 and 2 yr in this study population, suggesting that exposure to HCoV-229E, -NL63, -OC43, and -HKU1 may occur early in childhood.

To assess the relationship between anti-N-IgG and HCoV infection, we measured the IgG antibody in 361 serum samples from children with lower respiratory infections (Table 1). Of 246 samples from HCoV-negative patients, 124 (50.4%) had serologic evidence for past exposure of HCoV-229E, 142 (57.7%) for -NL63, 123 (50%) for -OC43, and 133 (54.1%) for -HKU1. However, among the 30 children who were HCoV-NL63-positive, only 7 (23.3%) were seropositive for anti-N-IgG. Similar results were found in serum samples from those who were positive for HCoV-NL63 (23.8%), -OC43 (18.4%), and -HKU1 (23.1%). Further analysis showed that IgG seropositive rates of HCoV-negative patients were significantly higher than those from HCoV-positive patients (χ^2 tests. $\chi^2 = 7.68$, $P = 0.0056$ for HCoV-NL63; $\chi^2 = 6.81$, $P = 0.00906$ for HCoV-229E; $\chi^2 = 11.98$, $P = 0.00054$ for HCoV-OC43; $\chi^2 = 7.84$, $P = 0.00511$ for HCoV-HKU1), suggesting that the low anti-N IgG level may be used as a predictive index for susceptibility to HCoV in a population.

In summary, our results suggest that the development of specific serologic diagnosis for HCoVs infection based on N proteins needs to take into consideration the cross-reactivities existing in the same subgroup. However, a common diagnostic platform for HCoVs should include a panel of phylogenetically distinct N proteins. Further, the anti-N IgG may serve as an indication of susceptibility to HCoV infections. Our study is informative for developing HCoV immunoassays and provides insights into the prevalence and pathologic roles of HCoVs.

Potentials conflicts of interest

No reported conflicts.

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Streptococcus suis meningitis in the Netherlands

Sir,

In 2008, a case report in *Journal of Infection* described four patients with *Streptococcus suis* meningitis in the Netherlands.[1] In 2006, a prospective nationwide cohort study of patients with community-acquired bacterial meningitis in the Netherlands was started.[1] In this new cohort study, 1732 episodes of bacterial meningitis were included between January 2006 and May 2015, of which 8 episodes were caused by *S. suis* in 7 patients.

The calculated annual incidence of *S. suis* meningitis in the Netherlands was 0.07 per 1,000,000 adult population. The median age was 54 years (range 28–77; Table 1). All cases concerned men who had professionally been in contact with pigs or pork: three pig farmers, two butchers and two abattoir workers. Headache was present in 6 out of 6 episodes (unknown in 2), fever and neck stiffness both in 5 out of 8, and altered consciousness in 7 out of 8 episodes. The classic meningitis triad consisting of fever, neck stiffness and an altered consciousness was present in 4 of 8 episodes. None presented with rash. All patients underwent a lumbar puncture and results of CSF analysis were abnormal in all. Blood culture was positive in 5 out of 7 episodes (71%) and serotype 2 was isolated in 4 out of 4 cases where serotype identification was performed (cases 1, 2, 3 and 8).

In seven episodes, initial antimicrobial therapy consisted of a cephalosporin (ceftriaxone in 5 episodes, cefotaxime in 2 episodes), combined with amoxicillin in 4 episodes (Table 1); after culture results became available, therapy was stepped down to penicillin in 3 episodes. One patient received penicillin monotherapy during admission. Adjunctive dexamethasone treatment according to the Dutch bacterial meningitis protocol (10 mg QID, for 4 days)[2] was administered in 7 of 8 episodes. Six out of seven patients (86%) developed hearing loss.

A literature search identified 6 articles describing 38 episodes of *S. suis* meningitis in 38 patients in the Netherlands (Table 2), occurring between 1988 and 2012.[1,3–7] One article described a patient who was also included in our cohort.[3] When combining the data with our patients, 45 different episodes were described in 44 patients since 1988. The median age was 50 years, and 39 out of 44 patients (89%) were male. In 41 of 44 (93%) cases the source of infection could be established: 16 were pig farmers, 11 were abattoir workers, 10 were butchers, and 4 had occasional contact with pigs or pork. Predisposing factors were present in 7 patients (16%) and consisted of cancer in 4 patients, and alcohol, immunosuppressive medication and splenectomy in the remaining 3 patients. Hearing loss developed in 28 out of 43 survivors (65%).

S. suis meningitis in the Netherlands occurs in patients with professional contact with pigs or pork. Whilst the calculated annual incidence of *S. suis* meningitis in our cohort was 0.07 per 1,000,000 adults, Schultsz and coworkers reported an estimated annual risk for developing *S. suis* meningitis in Dutch persons having regular contact with pigs of 3.4–5.6 per 100.000.[8] *S. suis* thus remains an important risk for persons having regular contact with pigs and infection is probably underreported, partly due to misidentification of *Streptococci*.[8] Invasive *S. suis* infections occur in pigs and humans.[9] In a recent study, it was shown that all cases of human *S. suis* infection in the Netherlands were caused by serotype 2: this study included the first three cases we described.[9] Infection in pigs was mainly caused by serotype 9.[9] The serotype causing *S. suis* meningitis in our cohort was not known in all patients, but no other serotypes but serotype 2 were found.

Hearing loss occurred in 86% of patients in our series, which is higher than described in patients with *S. suis* meningitis in Vietnam (50%),[10] and the earlier reviewed Dutch series (53%).[7] In a randomized clinical study on adjunctive dexamethasone in Vietnamese adults, dexamethasone was