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Letter to the Editors

Apoptosis inhibitor of macrophage differentiates bacteria from influenza or COVID-19 in hospitalized adults with community-acquired pneumonia

Dear Editor,

We read with interest the report by Li and colleagues in this journal who showed that serum smyloid A (SAA) was a biomarker of severe Coronavirus Disease and poor prognosis.[1] Rapidly and accurately differentiating between viral and bacterial infections in community-acquired pneumonia (CAP) is critical for prescribing appropriate therapy, especially in epidemic contexts such as influenza or coronavirus disease 19 (COVID-19).[2, 3]

We have reported that apoptosis inhibitor of macrophage (AIM, also called CD5L) production was elevated after bacterial infection,[4, 5] and it may predict mortality in the patients with sepsis.[6] Herein, we present evidence that AIM might be a biomarker in distinguishing between bacterial and viral infection in hospitalized adults with CAP.

In the first cohort study, 765 adult patients with CAP were enrolled at the Department of Respiratory and Critical Care Medicine of the First Affiliated Hospital of Chongqing Medical University from September 2017 to January 2020. All enrolled patients had clinical signs and radiographic evidence of CAP.[7] Influenza patients were defined as those with a positive virus result detected on viral nucleic acid testing. Bacterial infections were confirmed by microbiological evidence of bacterial infection. We classified patients into two groups based on laboratory test results: I, pure bacterial infection (detection of any bacteria other than virus); II, pure influenza (detection of influenza viruses A and B without co-detection of bacteria). CAP caused by mixed influenza and bacterial infection were excluded from analysis. Finally, 683 CAP patients did not meet the criteria, and 51 CAP patients had pure bacterial infection and 31 had pure influenza (Table 1). 62 sex- and age-matched healthy volunteers were recruited as healthy controls.

In the second cohort study from February 2020 to January 2021, 39 hospitalized patients with pure COVID-19 from the Department of Laboratory Medicine of Chongqing Public Health Medical Center, 47 hospitalized patients with pure bacterial infection from Department of Respiratory and Critical Care Medicine of the First Affiliated Hospital of Chongqing Medical University, and 51 healthy volunteers were enrolled (Table 1).

The following base-line variables were also recorded at enrollment: Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Sequential Organ Failure Assessment (SOFA) score, body temperature, white blood cells (WBC) count, serum levels of C-reactive protein (CRP) and procalcitonin (PCT), presence of shock, and the use of mechanical ventilation. The in-hospital mortality was also recorded. Serum levels of AIM were measured in duplicate using an enzyme-linked immunosorbent assay (ELISA) kit (Catalog# MBS064988; MyBioSource). This study was approved by the Research Ethics Committee of the First Affiliated Hospital of the Chongqing Medical University ((numbers 2021-187 and 2015–156).

Differences in continuous variables were estimated using the Mann–Whitney *U* test. Differences in categorical variables were calculated using the Fisher's exact or the Chi square test as appropriate. The ability of biomarker levels to discriminate between bacterial and viral infections was investigated by means of receiver operating characteristic (ROC) curve analysis. Area under the curve (AUC) was calculated. Sensitivity, specificity, and likelihood ratio (LR) values were calculated using AIM cut-points of 368 ng/ml, 430 ng/ml, and 567 ng/ml.

During the first study period, 51 CAP patients with pure bacterial infection and 31 with pure influenza were collected (Table 1). CAP patients with proven bacterial infection and those with influenza virus infection were not different in terms of demographics and chronic comorbidities. The severity of CAP on hospital admission was comparable between groups, as indicated by similar SOFA and APACHE-II scores, as well as similar percentages of shock and mechanical ventilation requirement. As expected, the levels of WBC, neutrophils, lymphocytes, CRP, and PCT were significantly higher in CAP with pure bacterial infection than those in CAP with pure influenza.

As shown in Fig. 1A, AIM concentrations were significantly elevated in the sera from CAP patients with influenza and those with bacterial infection than from healthy volunteers. Interestingly, serum AIM concentrations were significantly higher in bacterial group on the day of hospital admission when compared with influenza group. Next, we compared the AUC of AIM, PCT and CRP to differentiate bacterial infection from influenza by performing a ROC curve analysis (Fig. 1B), and the best AUC was observed for circulating AIM concentration at the day of hospital admission (0.90, 95% confidence interval [CI], 0.83–0.96). The AUC for PCT and CRP were, respectively 0.81 (95% CI, 0.71–90) and 0.73 (95% CI, 0.62–0.85). An AIM threshold of ≥430 ng/mL discriminated bacterial infection from influenza with a sensitivity of 80.6% (95% CI, 62.5%−92.5%) and specificity of 84.3% (95% CI, 71.4%–93.0%).

The second cohort study included 39 CAP patients with pure COVID-19 infection and 47 CAP patients with pure bacterial infection (Table 1). There were no significance differences in demographics, chronic comorbidities, severity of illness, and in-hospital morbidity and mortality between bacterial group and COVID-19 group. With regard to circulating AIM as shown in Fig. 1C, AIM concentrations were significantly higher in bacterial infection compared to COVID-19. Analysis of AUC [95% CI] showed that serum AIM concentration (0.97 [95% CI, 0.95–1.00]) robustly discriminated bacterial infection from COVID-19 in the patients with CAP on day of hospital admission (Fig. 1D).

PCT-guided therapy has successfully reduced antibiotics in selected populations of patients with respiratory infections.[7,8] How-

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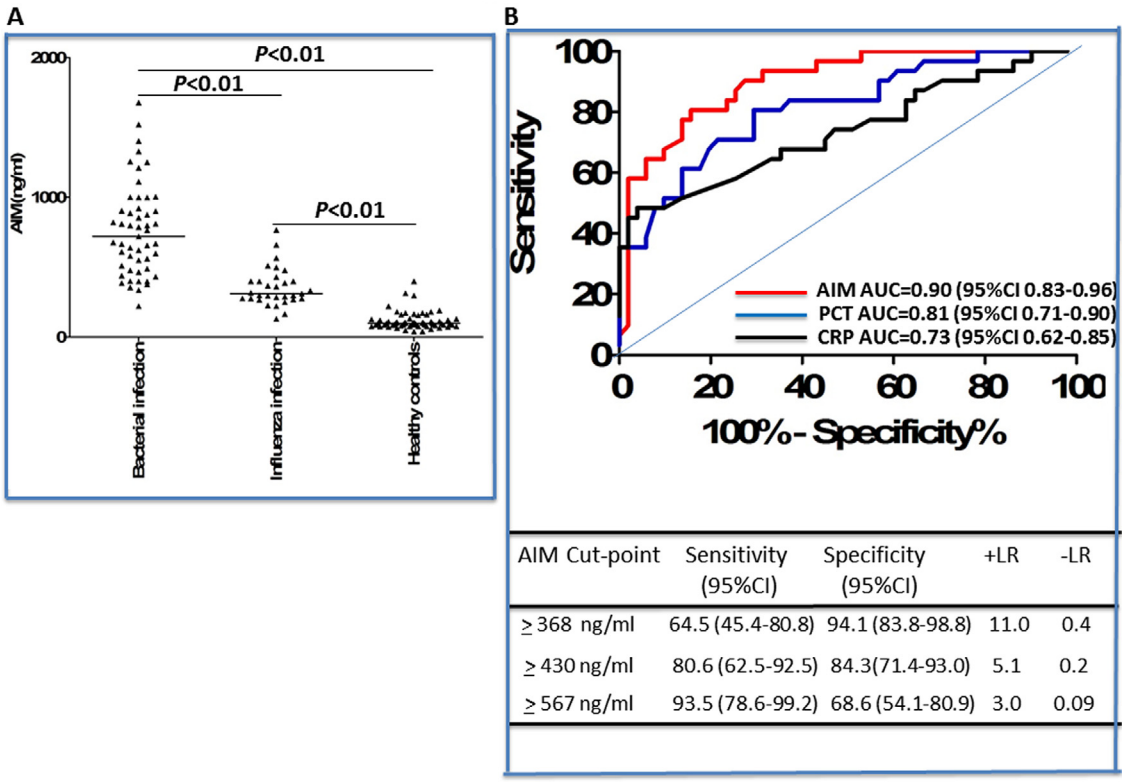

AIM Cut-point	Sensitivity (95%CI)	Specificity (95%CI)	+LR	-LR
≥ 368 ng/ml	64.5 (45.4-80.8)	94.1 (83.8-98.8)	11.0	0.4
≥ 430 ng/ml	80.6 (62.5-92.5)	84.3(71.4-93.0)	5.1	0.2
≥ 567 ng/ml	93.5 (78.6-99.2)	68.6 (54.1-80.9)	3.0	0.09

Fig. 1. Circulating AIM in adults hospitalized with community-acquired pneumonia and its utility to differentiate bacteria from influenza or COVID-19. (A) Levels of AIM in the serum from 51 CAP patients with pure bacterial infection, 31 CAP patients with pure influenza, and 62 healthy volunteers. Horizontal bars represent median values, and dots represent individual participants. $P<0.01$ when compared between groups denoted by horizontal bracket (Mann–Whitney *U* test). (B) Receiver operating characteristics curve for serum AIM, PCT and CRP in differentiating between bacterial infection and influenza in adults hospitalized with community-acquired pneumonia. AUC were 0.90 (95% CI, 0.83 to 0.96) for AIM, 0.81 (95% CI, 0.71 to 0.90) for PCT, and 0.73 (95% CI, 0.62 to 0.85) for CRP. Diagnostic test characteristics of AIM at selected cut-points (368 ng/ml, 430 ng/ml, 567 ng/ml) are displayed below. (C) Levels of AIM in the serum from 47 CAP patients with pure bacterial infection, 39 CAP patients with COVID-19, and 51 healthy volunteers. Horizontal bars represent median values, and dots represent individual participants. $P<0.01$ when compared between groups denoted by horizontal bracket (Mann–Whitney *U* test). (D) Receiver operating characteristics curve for serum AIM in differentiating between bacterial infection and COVID-19 in adults hospitalized with community-acquired pneumonia. AUC were 0.97 (95% CI, 0.95 to 1.00) for AIM. Abbreviations: AIM=apoptosis inhibitor of macrophage; AUC=area under the receiver operating characteristics curve; CI=confidence interval; COVID-19=coronavirus disease 19; CRP=C-reactive protein; LR=likelihood ratio; PCT=procalcitonin.

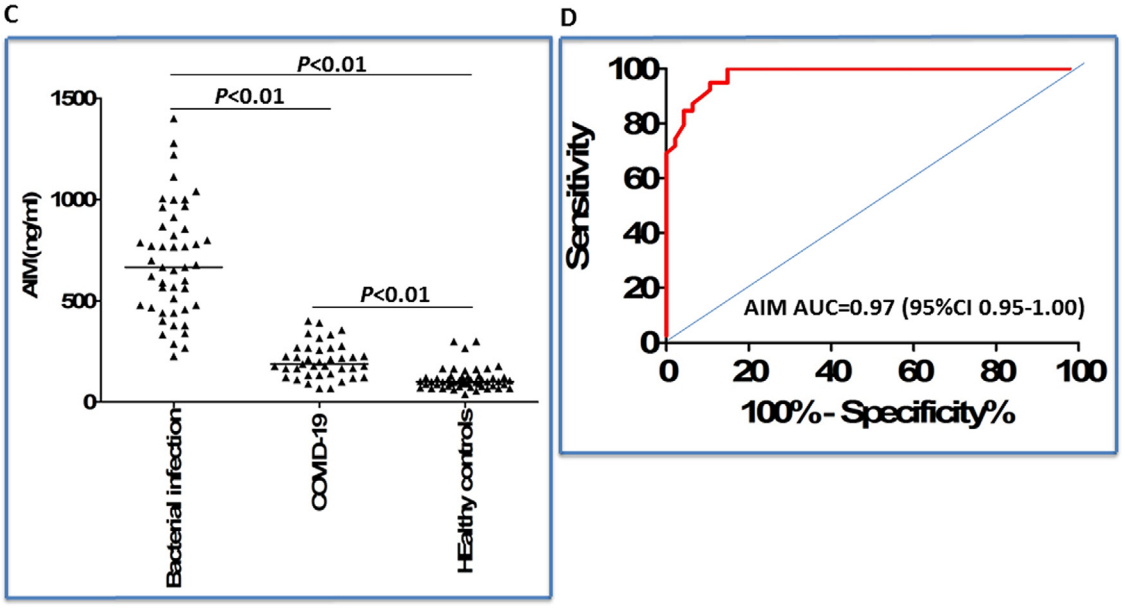

Fig. 1. Continued

Table 1.
Clinical characteristics of the study population.

Characteristic	2017–2019 cohort				2020–2021 cohort			
	Bacterial (n = 51)	Influenza (n = 31)	Healthy (n = 62)	P value	Bacterial (n = 47)	COVID-19 (n = 39)	Healthy (n = 51)	Pvalue
Demographic								
Age, median (IQR)	58 (49–71)	49 (35–67)	52 (38–69)	0.09	61 (44–77)	55 (33–74)	52 (36–71)	0.11
Female, n (%)	17 (33.3)	15 (48.1)	19 (30.6)	0.25	18 (38.3)	18 (46.1)	23 (45.1)	0.55
BMI, median (IQR)	27.6 (22.1–36.5)	24.0 (22.9–27.5)	22.6 (21.1–24.3)	0.37	25.9 (21.5–31.6)	24.9 (21.5–27.7)	22.9 (20.4–24.0)	0.76
Temp, median (IQR)	38.3 (37.7–39.3)	38.0 (37.4–39.0)	36.4 (36.0–36.9)	0.21	38.3 (37.6–39.4)	38.2 (37.7–39.2)	36.4 (36.0–36.9)	0.81
Chronic Comorbidities, n (%)								
Asthma	7 (13.7)	4 (12.9)	0 (0)	0.99	5 (10.6)	4 (10.2)	0 (0)	>0.99
COPD	5 (9.8)	3 (9.7)	0 (0)	>0.99	4 (8.5)	2 (5.1)	0 (0)	0.38
Diabetes	14 (27.4)	7 (22.6)	0 (0)	0.80	15 (31.9)	10 (25.6)	0 (0)	0.12
Hypertension	7 (13.7)	5 (16.1)	0 (0)	0.75	5 (10.6)	6 (15.4)	0 (0)	0.54
Heart failure	2 (3.9)	2 (6.5)	0 (0)	0.14	1 (2.1)	2 (5.1)	0 (0)	0.09
Kidney disease	4 (7.8)	2 (6.6)	0 (0)	0.88	4 (8.5)	2 (5.1)	0 (0)	0.10
Liver disease	3 (5.9)	2 (6.5)	0 (0)	0.99	3 (6.4)	2 (5.1)	0 (0)	0.63
Neurological disease	2 (3.9)	0 (0)	0 (0)	0.11	1 (2.1)	0 (0)	0 (0)	0.89
Laboratory values, median (IQR)								
WBC (10^9/L)	15 (8–21)	8 (5–12)	6 (5–8)	<0.01	14 (6–19)	9 (5–12)	6 (4–9)	<0.01
Neutrophils (10^9/L)	8 (5–15)	5 (2–10)	3 (2–5)	<0.01	8 (4–13)	6 (4–9)	3 (2–5)	<0.01
Lymphocytes (10^9/L)	3 (2–5)	1 (0.6–1.5)	2 (1.5–2.5)	<0.01	3 (2–5)	0.8 (0.6–1.1)	2 (1.5–2.5)	<0.01
CRP (mg/L)	79 (47–350)	55 (18–168)	NA	0.01	70 (51–220)	15 (4–120)	NA	<0.01
PCT (ng/ml)	1.1 (0.1–8.1)	0.6 (0.02–2.9)	NA	<0.01	0.9 (0.3–6.1)	0.05 (0.02–0.34)	NA	<0.01
Severity of illness, median (IQR)								
SOFA	5 (3–10)	4 (2–8)	NA	0.57	6 (2–9)	4 (2–10)	NA	0.22
APACHE II	14 (8–24)	10 (6–19)	NA	0.06	12 (5–22)	9 (3–19)	NA	0.05
In-hospital morbidity, n (%)								
Shock	7 (13.7)	3 (9.7)	NA	0.18	3 (6.3)	1 (2.6)	NA	0.19
Mechanical ventilation	10 (19.6)	5 (16.1)	NA	0.39	6 (12.8)	4 (10.2)	NA	0.46
In-hospital mortality, n(%)	6 (11.8)	3 (9.7)	NA	0.40	2 (4.3)	0 (0)	NA	0.14
Bacterial etiology, n (%)								
S.aureus	12 (23.5)	0 (0)	NA	NA	10 (21.3)	0 (0)	NA	NA
P.aeruginosa	10 (19.6)	0 (0)	NA	NA	11 (23.4)	0 (0)	NA	NA
H.influenzae	9 (17.6)	0 (0)	NA	NA	7 (14.9)	0 (0)	NA	NA
E.coli	8 (15.7)	0 (0)	NA	NA	8 (17.0)	0 (0)	NA	NA
S.pneumoniae	6 (11.8)	0 (0)	NA	NA	6 (12.8)	0 (0)	NA	NA
K.pneumoniae	5 (9.8)	0 (0)	NA	NA	4 (8.5)	0 (0)	NA	NA
M.catarrhalis	3 (5.9)	0 (0)	NA	NA	3 (6.4)	0 (0)	NA	NA
S.pyogenes	3 (5.9)	0 (0)	NA	NA	3 (6.4)	0 (0)	NA	NA
Serratia	1 (2.0)	0 (0)	NA	NA	0 (0)	0 (0)	NA	NA
Viral etiology, n (%)								
Influenza	0 (0)	31 (100)	NA	NA	0 (0)	0 (0)	NA	NA
COVID-19	NA	NA	NA	NA	0 (0)	39 (100)	NA	NA

Definition of abbreviations: APACHE II=acute physiology and chronic health evaluation II; BMI=body mass index; COPD= chronic obstructive pulmonary disease; COVID-19=coronavirus disease 2019; CRP=C-reactive protein; NA=not applicable; PCT=procalcitonin; SOFA=sequential organ failure assessment; Temp=temperature; WBC= white blood cell.
Differences in continuous variables were estimated using the Mann–Whitney U test. Differences in categorical variables were calculated using the Fisher's exact or the Chi square test as appropriate. P values indicate differences between bacterial and influenza or COVID-19 group. $P<0.05$ was considered statistically significant.

ever, some individual patients with bacterial pathogens did present to the hospital with low PCT levels,[7] and severe respiratory viral infection could induce PCT in the absence of bacterial pneumonia.[9] Therefore, clinicians cannot rely on PCT alone to guide antibiotic treatment decisions.

Our present findings are evidence of the value and accuracy of a rapid test for AIM in the blood to predict bacterial versus viral infection in the patients with CAP during influenza or COVID-19 epidemic. Higher levels of serum AIM at hospital admission were associated with increased probability of bacterial infections, suggesting suggest that AIM might be a useful adjunct in the etiologic assessment of patients hospitalized with CAP. Further study is required to evaluate its clinical utility in a larger cohort of pneumonia patients with suspected and proven infections.

Ethics approval and consent to participate

This study was carried out according to the principles of the declaration of Helsinki and approved by the Research Ethics Committee of the First Affiliated Hospital of the Chongqing Medical University ((numbers 2021-187 and 2015-156). Informed consent was obtained from all patients or their relatives prior to inclusion in the study.

Consent for publication

Not applicable.

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Declaration of Competing Interest

The authors declare no competing financial interests.

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References

1. Li H., Xiang X., Ren H., Xu L., Zhao L., Chen X., et al. Serum Amyloid A is a biomarker of severe Coronavirus Disease and poor prognosis. *J Infect* 2020;**80**(6):646–55.
2. Chow E.J., Doyle J.D., Uyeki T.M.. Influenza virus-related critical illness: prevention, diagnosis, treatment. *Crit Care* 2019;**23**(1):214.
3. Orkin A.M., Gill P.J., Ghersi D., Campbell L., Sugarman J., Emsley R., et al. Guidelines for reporting trial protocols and completed trials modified due to the COVID-19 pandemic and other extenuating circumstances: the CONSERVE 2021 statement. *JAMA* 2021;**326**(3):257–65.
4. Gao X., Yan X., Yin Y., Lin X., Zhang Q., Xia Y., et al. Therapeutic targeting of apoptosis inhibitor of macrophage/CD5L in sepsis. *Am J Respir Cell Mol Biol* 2019;**60**(3):323–34.
5. Gao X., Yan X., Zhang Q., Yin Y., Cao J.. CD5L contributes to the pathogenesis of methicillin-resistant Staphylococcus aureus-induced pneumonia. *Int Immunopharmacol* 2019;**72**:40–7.
6. Gao X., Liu Y., Xu F., Lin S., Song Z., Duan J., et al. Assessment of apoptosis inhibitor of macrophage/CD5L as a biomarker to predict mortality in the critically ill with sepsis. *Chest* 2019;**156**(4):696–705.
7. Self W.H., Balk R.A., Grijalva C.G., Williams D.J., Zhu Y., Anderson E.J., et al. Procalcitonin as a marker of etiology in adults hospitalized with community-acquired pneumonia. *Clin Infect Dis* 2017;**65**(2):183–90.
8. Niederman M.S., Baron R.M., Bouadma L., Calandra T., Daneman N., DeWaele J., et al. Initial antimicrobial management of sepsis. *Crit Care* 2021;**25**(1):307.
9. Gautam S., Cohen A.J., Stahl Y, Valda Toro P., Young G.M., Datta R., et al. Severe respiratory viral infection induces procalcitonin in the absence of bacterial pneumonia. *Thorax* 2020;**75**(11):974–81.

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RNA viral loads of SARS-CoV-2 Alpha and Delta variants in nasopharyngeal specimens at diagnosis stratified by age, clinical presentation and vaccination status

Dear Editor,

The SARS-CoV-2 Delta (B.617.2) variant has rapidly replaced the Alpha variant to become dominant in many European countries including Spain[1]. The Delta variant, which accumulates several Spike (S) mutations that increase virus binding to ACE2 (i.e. L452R and P681R), appears to outperform Alpha in terms of replication efficiency in the respiratory tract,[2] allegedly resulting in higher viral loads and enhanced transmissibility.[3,4] Interestingly, Ong et al.[5] in a cohort of adults among which Delta variant cases were underrepresented (8%), reported comparable SARS-CoV-2 Delta and Alpha RNA loads in nasopharyngeal specimens (NP) at the time of SARS-CoV-2 infection RT-PCR diagnosis. This observation clearly contrasts with data reported in other studies[9,6–9] which consistently show higher viral RNA loads for the Delta variant in the upper respiratory tract. None of the above studies[9,6–9] seemingly matched participants infected with the Delta or Alpha variant for presence of one or more of the following parameters, all with a potential impact on results: presence or absence of COVID-19 symptoms, demographics, time to testing from symptoms onset (or index case contact in asymptomatic subjects) or vaccination status. To gain further insight into this issue, we conducted a retrospective, observational study with a convenience sample of 545 participants of whom 295 (149 male; 231, adults; median age, 39 years; range, 1–93) and 250 (125 male; 128 adults; median age 22 years; range, 0–96) had been infected by the Alpha and Delta variants, respectively, as documented by whole-genome sequencing,[10] variant-specific RT-PCR (SARS-CoV-2 PCR Variant, Ascires, Sistemas Genómicos, Valencia, Spain), or inferred by S-gene target failure (SGTF) in the RT-PCR for the Alpha variant.[10] All cases due to the Alpha variant occurred in unvaccinated participants between February-May 2021, whereas 51/250 represented SARS-CoV-2 Delta breakthrough infections in fully vaccinated adults (diagnosed at least after 15 days after vaccine schedule completion) between May and July 2021. The study was approved by the INCLIVA Research Ethics Committee, and informed consent was waived due to its retrospective nature. SARS-CoV-2 RNA viral loads in NP were estimated by using the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, MS, USA) calibrated to the AMPLIRUN® TOTAL SARS-CoV-2 RNA Control (Vircell SA, Granada, Spain).[10]

We initially compared NP viral RNA loads in COVID-19 non-vaccinated individuals stratified by age (children, $\leq$18 years/adults) and time since symptoms onset (arbitrarily defined as 0–2, 3–7, or $\geq$ 8 days). As shown in Fig. 1, a trend towards higher viral RNA loads was seen for the Delta variant in both adults and children within 2 days after symptoms onset ($P = 0.27$ and $P = 0.26$, respectively), but not afterwards. Nonetheless, a wide range of viral RNA loads (3.-$\geq$ 11.0 $\log_{10}$ copies/ml) was observed in individuals in comparison groups. In participants asymptomatic at the time of RT-PCT testing, SARS-CoV-2 RNA loads were notably higher for the Delta than the Alpha variant, in both adults (median, 1.5 $\log_{10}$ higher) and children (median, 1.7 $\log_{10}$ higher) ($P = 0.08$ and $P = 0.01$, respectively).

Note that asymptomatic individuals in comparison groups were matched for time elapsed from diagnosis of the index case to RT-PCR testing (within 7 days, as per protocol). Unfortunately, no data were available regarding as to how asymptomatic infections evolved over time.

As can be seen in Fig. 2, the slope of the regression line best fitting SARS-CoV-2 RNA load decay in COVID-19 patients was slightly higher overall for the Delta than for the Alpha variant, irrespective of participant age (not shown). Although speculative, the above

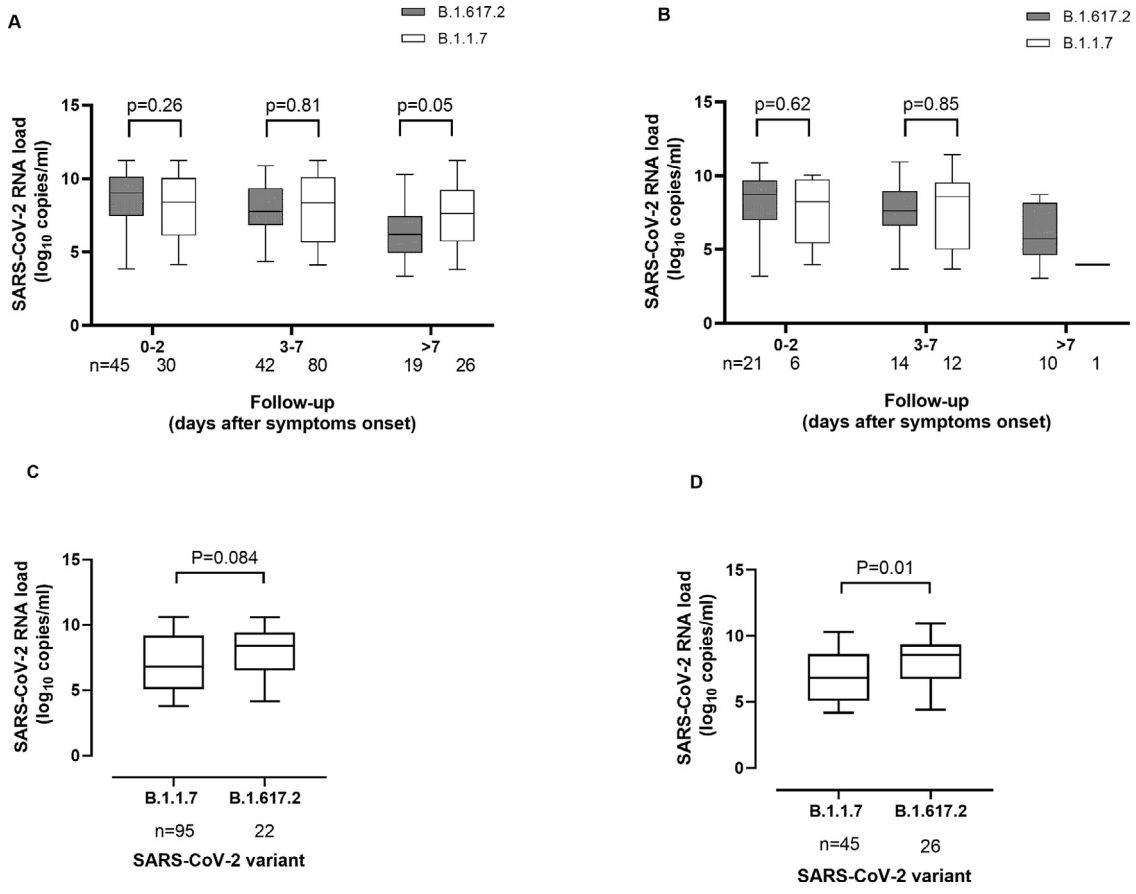

Fig. 1. Initial SARS-CoV-2 RNA loads in nasopharyngeal specimens from unvaccinated individuals infected by either Alpha (B.1.1.7) or Delta (B.1.617.2) variants. The Alpha lineage was confirmed by whole-genome sequencing in 108 cases, whereas in the remaining 230 it was inferred by S-gene target failure (SGTF) in the RT-PCR, as more than 95% of SGTFs detected in the Clínico-Malvarrosa Health Department belonged to that lineage within the timeframe of specimen collection (not shown). The Delta lineage was confirmed by whole-genome sequencing (n = 138) or variant-specific RT-PCR (n = 61). Whisker-plots of SARS-CoV-2 RNA loads in NP by time of sampling relative to symptoms onset are displayed separately for adults (A) and children (B). Whisker-plots of SARS-CoV-2 RNA loads in NP for asymptomatic participants are also shown separately for adults (C) and children (D). *P* values are shown for comparisons across groups.

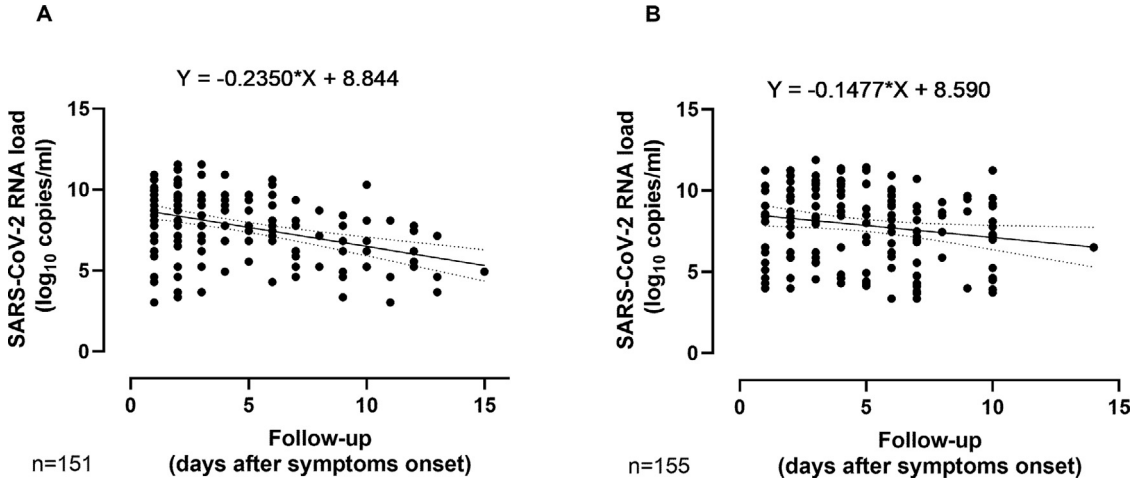

Fig. 2. Linear regression analysis of initial SARS-CoV-2 RNA loads from individuals infected with COVID by the Delta (A) and Alpha (B) variants according to time of sampling since symptoms onset. Regression lines best fitting SARS-CoV-2 RNA load decay are depicted.

data could be interpreted as suggesting that peak viral load in NP may be reached earlier in individuals infected by the Delta variant than the Alpha variant: even before symptoms onset, which favors increased transmissibility. In our view the data did not support a more extended period of viral shedding in the upper respiratory tract for the Delta variant, as previously suggested.[7]

We next compared SARS-CoV-2 Delta RNA loads in NP from non-vaccinated (n = 128) and fully vaccinated adults (n = 51) with Comirnaty® (n = 27), Spikevax® (n = 9), Janssen® (n = 10), or Vaxzebria® (n = 5). Time since full-dose vaccination was 51 days (range, 14 – 177). Overall, SARS-CoV-2 RNA load was found to be higher in non-vaccinated (n = 128; median, 8.1 $\log_{10}$ copies/ml;

range, 3.4–11.6) than vaccinated individuals ($n = 51$; median, 7.8 $\log_{10}$ copies/ml; range 3.0–11.2), although without reaching statistical significance ($P = 0.31$). When considering patients with COVID-19 for the analysis (matched for time since symptoms onset: median, 3 days in both groups), a clear trend ($P = 0.12$) towards higher viral RNA loads was observed in non-vaccinated individuals (8.1 $\log_{10}$ copies/ml; range, 3.3–11.6) compared with vaccinated participants (median, 7.4 $\log_{10}$ copies/ml; range, 3.3–11.2).

In contrast, asymptomatic vaccinated and unvaccinated individuals displayed rather similar viral RNA loads (median, 8.7 $\log_{10}$ copies/ml; range, 3–0–10.9, and median, 8.4 $\log_{10}$ copies/ml; range, 4.0–10.6; $P = 0.85$). Vaccinated and unvaccinated participants were matched for sex ($P = 0.1$, but not age, $P = < 0.01$). Contradictory data[9,8] have been published on this issue, which are likely explained by between-study differences regarding time of RT-PCR testing.

Finally, we showed that initial SARS-CoV-2 RNA loads in NP for the prototypical B.1.617.2 variant ($n = 138$) and recently emerged subvariants ($n = 47$; AY.4, $n = 29$, AY.12, $n = 9$, AY.5, $n = 5$, AY.9, $n = 3$ and AY.3, $n = 1$) were quite similar when considered in combination (median, 8.7 log10 copies/ml; range, 4.6–11.6 vs. median, 8.4 log10 copies/ml; range, 4.0–12.1; $P = 0.20$). Comparison groups were matched for sex ($P = 0.61$), age ($P = 0.34$), development of COVID-19 ($P = 0.44$), time to NP sampling for both symptomatic and asymptomatic cases ($P = 0.80$) and vaccination status ($P = 0.20$). Of note, no B.1.617.2 subvariants seemed to carry additional spike mutations (K417N, Y145H and A222V) than might confer partial resistance to vaccine-elicited neutralizing antibodies. In summary, we found substantially higher NP initial viral loads for the Delta than the Alpha variant, in particular during the asymptomatic phase of the infection. Together with the overall lack of significant differences observed between viral loads in vaccinated and unvaccinated Delta-infected participants, this may help explain the epidemiological behavior of the Delta variant.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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References

[1]. Data on SARS-CoV-2 variants in the EU/EEA. https://www.ecdc.europa.eu/en/publications-data/data-virus-variants-covid-19-eueea1. Accessed October 21, 2021.

[2]. Mlcochova P., Kemp S.A., Dhar M.S., Papa G., Meng B., Ferreira I.A.T.M., et al. SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. *Nature* 2021 Sep 6. doi:10.1038/s41586-021-03944-y.

[3]. Campbell F., Archer B., Laurenson-Schafer H., Jinnai Y., Konings F., Batra N., et al. Increased transmissibility and global spread of SARS-CoV-2 variants of concern as at June 2021. *Eurosurveillance* 2021;**26**(24):2100509 Jun.

[4]. Teyssou E., Delagrèverie H., Visseaux B., Lambert-Niclot S., Brichler S., Ferre V., et al. The Delta SARS-CoV-2 variant has a higher viral load than the Beta and the historical variants in nasopharyngeal samples from newly diagnosed COVID-19 patients. *J Infect* 2021;**83**(4):e1–3 Oct.

[5]. Ong S.W.X., Chiew C.J., Ang L.W., Mak T.M., Cui L., Toh M.P.H.S., et al. Clinical and virological features of SARS-CoV-2 variants of concern: a retrospective cohort study comparing B.1.1.7 (Alpha), B.1.315 (Beta), and B.1.617.2 (Delta). *Clin Infect Dis* 2021:ciab721 Aug 23.

[6]. Wang Y., Chen R., Hu F., Lan Y., Yang Z., Zhan C.. Transmission, viral kinetics and clinical characteristics of the emergent SARS-CoV-2 Delta VOC in Guangzhou, China. *EClinicalMedicine* 2021;**40**:101129 Oct.

[7]. Luo C.H., Morris C.P., Sachithanandham J., Amadi A., Gaston D., Li M., et al. Infection with the SARS-CoV-2 delta variant is associated with higher infectious virus loads compared to the alpha variant in both unvaccinated and vaccinated individuals. *medRxiv* 2021 Aug 20:2021.08.15.21262077.

[8]. Riemersma K.K., Grogan B.E., Kita-Yarbro A., Halfmann P., Kocharian A., Florek K.R., et al. Shedding of infectious SARS-CoV-2 despite vaccination. when the delta variant is prevalent - Wisconsin, july 2021. *medRxiv* 2021 August 11. doi:10.1101/2021.07.31.21261387.

[9]. Blanquart F., Abad C., Ambroise J., Bernard M., Cosentino G., Giannoli J.M., et al. Characterisation of vaccine breakthrough infections of SARS-CoV-2 Delta and Alpha variants and within-host viral load dynamics in the community, France, June to July 2021. *Eurosurveillance* 2021;**26**(37):2100824 Sep.

[10]. Costa R., Bueno F., Giménez E., Bracho A., Albert E., Carretero D., et al. Initial viral load and decay kinetics of SARS-CoV-2 lineage B.1.1.7 in the upper respiratory tract of adults and children. *J Infect* 2021;**83**(4):496–522 Oct.

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COVID-19 and alarming dengue co-epidemics in the dilapidated healthcare system in Pakistan: Where to focus!

Dear Editor,

Coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2). The WHO classified it a worldwide pandemic. COVID-19, a new virus, has been identified in 221 developing and developed nations.[1,2] Globally, there are 239,973,250 confirmed COVID-19 cases, 4890,002 deaths, and 217,312,219 recovered cases. In Pakistan, the overall number of confirmed cases has reached 1,261,685, with 28,201 fatalities.

Dengue outbreaks have occurred frequently in Pakistan during the previous three decades, perpetuating dengue endemicity. The current COVID-19 pandemic coincides with an outbreak of dengue fever, a viral infection caused by *Aedes albopictus* and *Aedes aegypti* mosquito vectors that pose a substantial threat to human health. As a result of the COVID-19 pandemic, dengue-endemic countries in Latin America and Southeast Asia have a heightened risk of infection overlap.[3] Each year, more than a hundred countries face a pandemic burden of 50 million dengue cases.[4] Dengue fever was verified in 132,977 cases and 588 deaths in Pakistan over the previous decade, according to the Ministry of National Health Services.[5] In Pakistan, confirmed dengue pandemic cases were 52,485 in 2019, including 91 deaths and 4024 in 2020.[6] During the COVID-19 pandemic of 2021, just 2875 cases were reported until September, with 678 occurring in Lahore, the capital of Punjab Province. During the rainy season, dengue disease is growing rapidly in Lahore. The number of actual dengue cases may be higher than reported in the literature, as many of the patients were asymptomatic. Dengue infection and COVID-19 are co-related by laboratory features and clinical demonstration, which can lead to misdiagnosis. Patients co-infected with dengue and COVID-19 show initial symptoms like rash, high fever, and headache, while as diseases progress, the symptoms can be distinguished separately. There is an increase in dengue cases in Pakistan because COVID-19 hype has diverted focus and led to a lack of supervision.[7] Dengue cases normally start to appear post monsoon season and are highest in September and October in Pakistan. Since last year dengue cases have been occurring along with COVID cases, and even co-infection can be seen in certain patients like other dengue-endemic countries, for example, South East Asia, Brazil, Thailand, India, France, and Malaysia.

The co-infection of COVID-19 and dengue cases reported in Singapore had similar symptoms of headache and high fever. In Pakistan, cases of co-infection have been reported, and a study showed that patients suffering from dengue and COVID-19 have more mortality rate than patients who only have COVID-19.[8] Similarly, COVID-19 mono-infected patients have different biochemical and hematological parameters than those who are co-infected. For example, co-infected patients suffered from severe thrombocytopenia while mono-infected patients did not. There was also an increase in creatine phosphokinase, urea, prothrombin time, creatinine, alanine aminotransferase, and bilirubin concentrations in co-infected patients.[9]

Since 2010, Pakistan has been witnessing a dengue fever outbreak, which has resulted in 16 580 confirmed cases and 257 fatalities in Lahore, and almost 5000 cases and 60 deaths in the rest of the nation. Khyber Pakhtunkhwa, Punjab, and Sindh are the three provinces affected by the disease[1]. Dengue fever is native to Balochistan's coastal regions but has historically been more prevalent in Khyber Pakhtunkhwa Province. Pakistani authorities recorded 3442 dengue fever cases in 2017, almost 3200 in 2018, about 24,547 in 2019, and 3442 in 2020.[2] In the first half of the year, more than 820 reported cases have been reported, with 687 of those occurring in Lahore. In 2019, more than 8670 instances with a total of over 50,000 were recorded in Punjab.[3] During the research period (2006–2017), 2011 was Pakistan's most destructive year. In a single year, Lahore had almost 17,000 confirmed cases and 290 fatalities.[4] Between 1995 and 2019, over 147 200 dengue illness cases and over 800 fatalities.[5] As of September 30, 2021, the total number of confirmed dengue cases in Lahore has reached 1180.[6] On November 6, 2019, the country's dengue outbreak hit a new record, with over 44,000 individuals afflicted and 66 deaths. In 2011, 27,000 individuals were infected, yet the illness claimed nearly six times as many lives as in 2019, when 370 people died.[9] According to the Ministry of National Health Services, Regulations, and Coordination, the number of dengue cases increased about 15-fold from 3204 cases in 2018 to 47,120 cases in 2019, although the true figure is expected to be considerably higher. Similarly, 416 confirmed dengue cases had been reported considerably earlier in 2020, during the post-monsoon months.[7,9] There is a likelihood of an increase in dengue cases due to the COVID-19 lockdown; people remain at home to avoid contracting the fatal virus, and dengue vector mosquitos are bred in the clean waters of home gardens. Even if they get fever due to dengue, many are afraid to contact a physician for fear of contracting COVID-19.[10] In a study by Siddique A. at Islamabad's Holy Family Hospital, 20 patients admitted to the intensive care unit with severe respiratory disorders were sent to the virology department for real-time reverse transcription-polymerase chain reaction (RT-PCR) diagnosis. All twenty patients tested positive for COVID-19, and five were co-infected with dengue and COVID-19. Fever was seen in 100% of co-infected individuals and in 80% of mono-infected patients. Both groups had low lymphocyte, neutrophil, platelet, and white blood cell counts and concentrations. Mono-infected patients survived, while 60% of co-infected individuals did not, indicating a greater death risk in co-infected patients.[9] A developing countries like Pakistan having deteriorated health system two pandemics co-existing is a great threat, especially dengue infection that emerges and re-emerges every few years. Healthcare authorities should take the necessary measures against the spread of epidemics like COVID-19 and dengue and spread awareness about their co-existence in one patient to restrain the exposure and transmission ways.

Declaration of Competing Interest

The authors declare that, they have no conflict of interests.

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References

1. WHO. World health organization corona virus disease (COVID 19) outbreak situation. 2021 [cited 2021 October 24]. Available from: https://www.who.int/emergencies/diseases/novelcoronavirus-2019.
2. Rahman, M.S.M., Hossain S.M., et al.. COVID-19 in Bangladesh: measures for containment. 2020;46(1):1–2.
3. Ridwan R.. COVID-19 and dengue: a deadly duo. *Trop Doct* 2020;**50**(3):270–2.
4. Shirin T.. Dengue fever in Bangladesh. *Bangladesh Med Res Counc Bull* 2019;**45**(3):214–15.
5. Rana M.S., Alam M.M., Salman M., Ikram A.. Prevention and control of escalating dengue epidemics in Pakistan. *J Med Virol* 2020;**92**(8):927–8 PubMed PMID: 31792995. Epub 2019/12/04. eng.
6. NIH. National institute of health Pakistan weekly epidemiological report 2021,Vol 3, page 1-7 [cited 2021 October 14]. Available from: https://www.nih.org.pk/wp-content/uploads/2021/04/14-FELTP-Pakistan-Weekly-Epidemiological-Report-Mar-28-Apr-03-2021.pdf
7. Haqqi A., Awan U.A., Ali M., Saqib M.A.N., Ahmed H., Afzal M.S.. COVID-19 and dengue virus co-epidemics in Pakistan: a dangerous combination for overburdened healthcare system. *J Med Virol* 2020;**3**(11):1–7.
8. Rana M.S., Alam M.M., Ikram A., Zaidi S.S.Z., Salman M., Khurshid A.. Co-circulation of COVID-19 and dengue: a perspective from Pakistan. *J Med Virol* 2021;**93**(3):1217–18 PubMed PMID: 32990999. Pubmed Central PMCID: PMC7537270. Epub 2020/09/30. eng..

9. Saddique A., Rana M.S., Alam M.M., Ikram A., Usman M., Salman M., et al. Emergence of co-infection of COVID-19 and dengue: a serious public health threat. *J Infect* 2020;**81**(6):e16–ee8 PubMed PMID: 32800797. Epub 2020/08/12. eng.
10. Nacher M., Douine M., Gaillet M., Flamand C., Rousset D., Rousseau C., et al. Simultaneous dengue and COVID-19 epidemics: difficult days ahead? *PLoS Negl Trop Dis* 2020;**14**(8):e0008426.

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Wild bird-origin H3N8 avian influenza virus exhibit well adaptation in mammalian host

Dear Editor

A recent article in this Journal has reported that wild bird-origin H5N6 avian influenza virus show transmission ability in mammalian host.[1] Actually, wild birds play an essential role in the emergence, evolution and spread of zoonotic avian influenza viruses. For example, many novel emerged human infected H7N9, H7N4, H10N8 virus were closely related to wild bird-origin influenza virus.[2–4] Wild birds form a large gene pool for influenza A viruses in nature, such that 16 HA and 9 NA subtypes can be found in this reservoir.[5] Among them, H3N8 virus was one of the most commonly found subtype in wild birds.[6] Moreover, sporadic cross-species transmission events of the H3N8 influenza virus have been reported for various species such as pigs, dogs, horses, seals and donkeys.[7–9] This suggests that H3N8 virus can easily cross species barriers and may pose a potential threat to animal and human health and has the potential to trigger the epidemic of virus in the population.

To investigate the viral evolution in wild bird reservoirs, we undertook surveillance in Xinjiang Province, China, from 2017 to 2020 along East Africa-West Asia migratory bird flyway. Totally 38 of 592 fecal sample collected were positive for influenza virus. Then the viral RNAs of these positive samples were extracted and the reverse transcription was subsequently carried out using the and subtype was determined using PCR. One sample isolated from a mallard duck was identified as H3N8 and designated A/Wide Bird/Ebinur Lake-xinjiang/47/2017(H3N8)(XJ47).

Each segment of this sample was amplified according to Hoffmann's approach.[10] The amplified products were sequenced and phylogenetic analysis was performed. Phylogenetic analysis of the HA gene showed that this virus belong to the Eurasian lineage and markedly distinct from classical equine influenza virus lineage and North American avian influenza virus lineage(Fig. S1).A BLAST search in the GenBank database demonstrated a high nucleotide identity of the XJ47 virus with multiple subtypes circulating in wild and domestic birds in Asian countries (Table 1). Both the HA and NA gene of the XJ47 virus were closest to that of A/duck/Mongolia /179/2015(H3N8), with 98.07% and 99.36% identity, respectively. This genetic relationship indicates that the surface genes of H3N8 virus might have been introduced from Mongolia through long-term migration of wild birds. Whereas, the internal genes of the XJ47 virus were most closely related to other subtypes from other countries (Table 1). These indicated that XJ47 virus is a novel reassortant H3N8 virus through the complex reassortment of multiple AIV subtypes in the wild bird population.

The amino acid residues at the cleavage site (340–348) of the HA molecule are PEKQTR↓GLF with one basic amino acid, which is characteristic of low pathogenic AIV. And the amino acid residues at the receptor binding site in the HA protein are Q226 and G228, which preferentially bind to an avian-origin receptor.None of the substitutions for mammalian adaptation in PB2 such as A588V, G590S, Q591R, E627 K or D701N[11,12] were found. However, several putative mammalian adaptive mutation in PB1, PA was occurred in this H3N8 virus(1 mutation in PB1 protein and 5 mutations in PA protein)(Table S1).

The switch of receptor binding specificity from a2,3-linked to a2,6-linked salid acid is a critical determinant for avian influenza gain the cross-species transmission to human. Subsequently the receptor-binding specificity of the XJ47 virus was determined by HA assays with chicken red blood cells treated with or without α2,3 sialidase. The similar HA titers demonstrated that the virus can bind to both avian-type and human-type receptors (Fig. 1A).

Finally, to evaluate the pathogenicity of the virus to mammals, Balb/c mice were intranasally inoculated with XJ47 virus at a dose of 10^6 EID_{50}, weight loss was monitored for 14 dp.i. and viral titres in lungs and nasal turbinate tissues were determined on days 3 and 5 dp.i. The result suggested that XJ47 virus caused significant weight loss (15%) compared with uninfected control (Fig. 1B) and the virus could be detected in the lung and nasal turbinate tissues of infected mice (Fig. 1C,D). These results indicated that the H3N8 virus Xj47 obtain the replication ability and exhibit pathogenicity to mammalian host species.

In summary, this study has demonstrated that a novel reassortant avian influenza A(H3N8) virus XJ47 isolated from wild birds obtained enhanced α2,6 receptor binding and exhibited a well adaptation in mammals. It may pose a potential threat to animal and human health and inform us that continuous surveillance of the avian influenza virus,especially the H3 subtype of AIV circulating in wild bird reservoirs is warranted.

Declaration of Competing Interest

All authors: No conflicts.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.12.014.

Table 1
Nucleotide sequence identities between the H3N8 virus XJ47 and the closest homologs in the GenBank database.

Gene	Virus	GenBank accession no.	Subtype	Identity(%)
HA	A/duck/Mongolia/179/2015	LC121310	H3N8	98.07
NA	A/duck/Mongolia/179/2015	LC121310	H3N8	99.36
PB2	A/duck/Mongolia/769/2015	LC121449	H4N6	100
PB1	A/Mallard/Ukraine/Kurganskoe-7–16–11/2011	MW132991	H2N3	97.76
PA	A/aquatic bird/South Korea/sw003/2016	MG770151	H1N1	98.23
NP	A/duck/Bangladesh/34,192/2017	MH791548	H3N1	98.60
M	A/duck/Hubei/ZYSYG1/2015	KY415650	H6N2	99.60
NS	A/duck/Assam/DUOR1512100030/2015	MT272393	H3N8	98.99

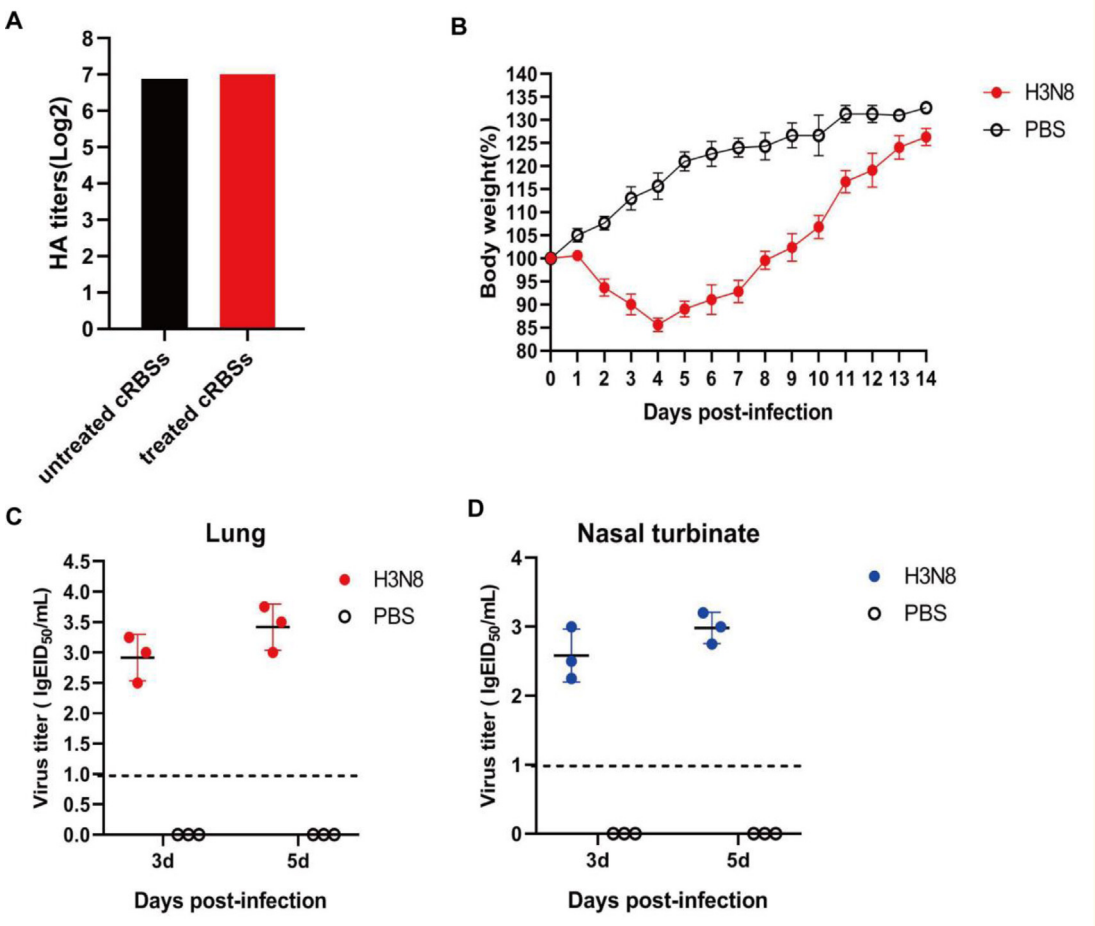

Fig. 1. Agglutination activity and pathogenicity of the wild bird-origin H3N8 virus in mice. (A) Agglutination activities of the H3N8 virus with chicken erythrocytes treated with or without α2,3 sialidase.(B)Body weight change differences between mice inoculated with XJ47 virus and PBS, and viral titers from lung(C) and nasal turbinate tissues(D) of mice were determined after infected with the XJ47 virus at 3 dpi and 5dpi.To evaluate the pathogenicity of avian H3N8 viruses, group of 10 6–8-week-old BALB/c mice were inoculated with 50 µL of 10^6 EID_{50} of the H3N8 virus and weight loss was monitored for 14 dp.i. At 3 and 5 dp.i., lungs and nasal turbinate tissues were collected from three mice per group and viral titres were determined in homogenates by EID_{50} analysis. Data are given as mean ± standard deviations (SDs). The dashed lines indicate the lower limit of detection.

References

1. Xiang B., Chen L., Xie P., Lin Q., Liao M., Ren T.. Wild bird-origin H5N6 avian influenza virus is transmissible in guinea pigs. *J Infect* 2020;**80**(5):e20–2.
2. Tong X., Weng S., Xue F., Wu X., Xu T., Zhang W.. First human infection by a novel avian influenza A(H7N4) virus. *J Infect* 2018;**77**(3):255–7.
3. Qi W., Zhou X., Shi W., Huang L., Xia W., Liu D., et al. Genesis of the novel human-infecting influenza A(H10N8) virus and potential genetic diversity of the virus in poultry, China. *Eurosurveillance* 2014;**19**(25):50–62.
4. Gao R., Cao B., Hu Y., Feng Z., Wang D., Hu W., et al. Human infection with a novel avian-origin influenza a (H7N9) virus. *New Engl J Med* 2013;**368**(20):1888–97.
5. Olsen B., Munster V.J., Wallensten A., Waldenstrom J., Osterhaus A.D., Fouchier R.A.. Global patterns of influenza a virus in wild birds. *Science* 2006;**312**(5772):384–8.
6. Henaux V., Samuel M.D., Dusek R.J., Fleskes J.P., Ip H.S.. Presence of avian influenza viruses in waterfowl and wetlands during summer 2010 in California: are resident birds a potential reservoir? *PLoS ONE* 2012;**7**(2):e31471.
7. Anthony S.J., St L.J., Pugliares K., Ip H.S., Chan J.M., Carpenter Z.W., et al. Emergence of fatal avian influenza in New England harbor seals. *MBio* 2012;**3**(4):e112–66.
8. Tu J., Zhou H., Jiang T., Li C., Zhang A., Guo X., et al. Isolation and molecular characterization of equine H3N8 influenza viruses from pigs in China. *Arch ViroL* 2009;**154**(5):887–90.
9. Qi T., Guo W., Huang W., Dai L., Zhao L., Li H., et al. Isolation and genetic characterization of H3N8 equine influenza virus from donkeys in China. *Vet Microbiol* 2010;**144**(3–4):455–60.
10. Hoffmann E., Stech J., Guan Y., Webster R.G., Perez D.R.. Universal primer set for the full-length amplification of all influenza A viruses. *Arch Virol* 2001;**146**(12):2275–89.
11. Xiao C., Ma W., Sun N., Huang L., Li Y., Zeng Z., et al. PB2-588V promotes the mammalian adaptation of H10N8, H7N9 and H9N2 avian influenza viruses. *Sci Rep* 2016;**6**:19474.

12. Schrauwen E.J., Fouchier R.A.. Host adaptation and transmission of influenza a viruses in mammals. *Emerg Microbes Infect* 2014;**3**(2):e9.

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EK1 with dual Q1004E/N1006I mutation: a promising fusion inhibitor for the HR1 domain of SARS-CoV-2

Dear Editor,

This journal has previously published commentaries on the consequences and severity of coronavirus disease 2019 (COVID-19) and the urgent need for novel therapy[1]. The epidemic of COVID-19 has posed a great threat to many aspects including human health and the global economy. However, there is still no effective strategy to combat this disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the number of infections by this virus is still increasing, as recorded in an online website: https://www.worldometers.info/. It is reported that spike (S) protein is the main player of SARS-CoV-2 invasion into the host, and its structure can be divided into two domains (S1 and S2) that perform different functions[2]. The S1 domain mainly promotes the binding of SARS-CoV-2 to the host through the interaction between its receptor binding domain (RBD) and angiotensin-converting enzyme 2 (ACE2), and the interaction mechanism between RBD and ACE2 has been discussed in detail[3,4]. After the binding of S1 domain to ACE2, the S2 domain will initiate the fusion of the viral and host cell membranes. During this fusion process, the trimeric heptad repeat 1 (HR1) region of S2 domain will interact with the trimeric heptad repeat 2 (HR2) region of S2 domain to form a stable six-helix bundle (6-HB) structure to facilitate the close proximity of the membrane[5]. The 6-HB core plays a crucial role in the membrane fusion of the coronavirus and the host. In addition, HR1, HR2 and their interaction mode are conserved in various coronaviruses. Thus, HR1 and HR2 are considered to be promising targets for the development of fusion inhibitors against coronaviruses. Recently, Xia et al. have proved that the fusion inhibitor EK1 has inhibitory activity against SARS-CoV-2, and it can inhibit the formation of 6-HB fusion core, thereby hindering the fusion of viral and host cell membranes[5,6]. The confirmation of this inhibitor provides novel ideas and insights for the future design of inhibitors against SARS-CoV-2.

Our focus here is to enhance the inhibition ability of EK1 through further optimization, and the binding affinity of the newly optimized inhibitors to HR1 domain of SARS-CoV-2 is stronger than that of EK1 to HR1. On the basis of the inhibitor EK1, we conducted a comprehensive screening of potential inhibitors for the HR1 domain of SARS-CoV-2 through a combination of residue mutation, molecular dynamics (MD) simulation and binding energy calculation.

The trimeric structure of HR1-EK1 complex was acquired from the protein data bank (PDB ID: 7C53), as shown in Fig. 1A. The inhibitor EK1 is displayed in cartoon mode, and three EK1s in the trimer are represented by chains A, B and C, respectively. To explore the mutant residues that can enhance the binding affinity of EK1 to HR1, all the residues on EK1 in the trimer were mutated to 19 residues other than itself, and the changes in binding affinity resulting from 1824 possible mutations were preliminarily predicted by mCSM-PPI2[7] (Fig. 1B). Among these mutations, the mutations Q1004E and Q1004D in chain A, Q1004E and Q1004D in chain B, and Q1004Y and N1006I in chain C could more significantly enhance the binding ability of EK1 to HR1, resulting in an increase in binding affinity by more than 1 kcal/mol. Although the performance of mCSM-PPI2 is better than that of previously developed tools including FoldX, mCSM-PPI2 still has certain shortcomings. For example, it can only predict single mutation, not multiple mutations. In the following work, the binding properties of trimeric HR1 to EK1s with single mutation (Q1004E, Q1004D, Q1004Y and N1006I) and dual mutation (Q1004E/N1006I, Q1004D/N1006I and Q1004Y/N1006I) were deeply analysed to overcome this problem.

MD simulation has been proven to be an effective tool for exploring the binding properties of receptors and ligands[8,9]. MD simulations of the above complexes were performed mainly by the following steps: (1) preparing the system, including adding hydrogen atoms and counterions, and setting the force field; (2) performing optimization, heating and dynamic equilibrium; (3) running long-time (200 ns) MD simulations without any restrictions. Through root mean square deviation (RMSD) analysis, it is found that all systems have reached equilibrium after 120 ns MD simulations, and their RMSD values are basically less than 2.5 Å, indicating that all systems are already in a stable state and can be used for subsequent calculations (Fig. 1C).

To investigate the binding ability of the wild-type (WT)/mutant EK1s to HR1, molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) and solvated interaction energy (SIE) methods were selected to predict the binding free energy (ΔG) between them. First, 10,000 conformations were extracted from the equilibrium MD trajectory with a conformation interval of 1 frame for calculation. From the calculated results of the MM-PBSA method (Fig. 1D), it can be seen that all the selected single mutations (Q1004E, Q1004D, Q1004Y and N1006I) on EK1s can enhance the binding affinity of EK1 to HR1, which is consistent with the predicted results of mCSM-PPI2. Notably, the binding affinity of the mutant EK1s with dual mutation (Q1004E/N1006I, Q1004D/N1006I and Q1004Y/N1006I) to HR1 was also significantly enhanced compared to that of WT EK1 to HR1, and the mutant EK1 with Q1004E/N1006I showed the strongest binding ability to HR1. The similar results was also obtained by the SIE method (Fig. 1F). Next, different conformational intervals were applied to calculate the binding free energy to confirm these results (Fig. 1E and 1G). It is found that the mean of the binding free energies calculated at different intervals was largely consistent with the result shown in the above figures (Fig. 1D and 1F), which further suggests that the selection of the conformation is reasonable. Finally, the hotspot residues that promote the binding of WT/mutant EK1s to HR1 were also found through free energy decomposition and hierarchical clustering analyses (Fig. 1H). As shown in Fig. 1H, all EK1s in the trimer have similar clustering results. According to the energy contribution of each residue, the residues on EK1 are mainly divided into three clusters (*a*, *b* and *c*), and the residues

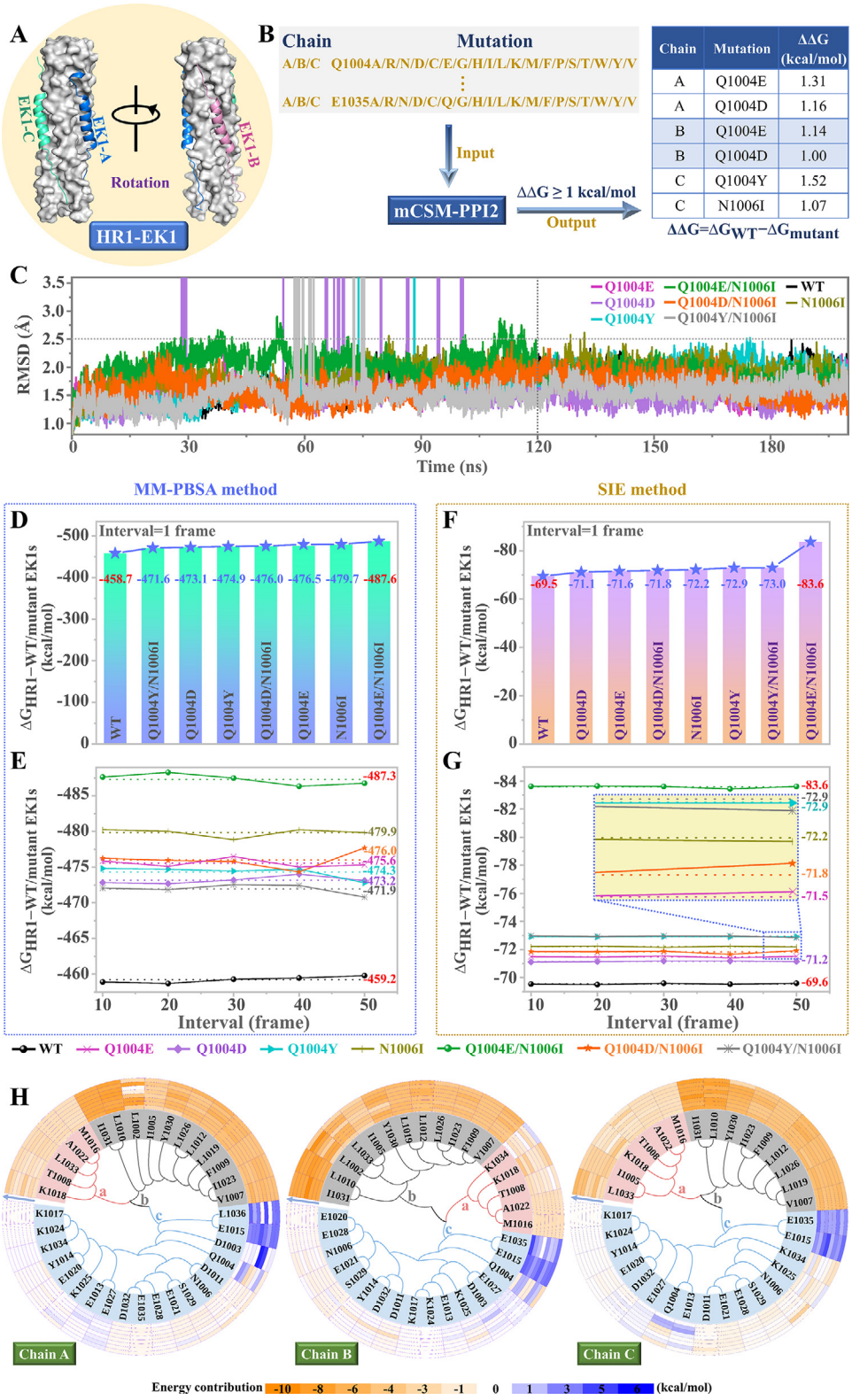

Fig. 1. Binding characteristics of WT/mutant EK1s to HR1. (A) The trimeric structure of HR1-EK1 complex, in which HR1 and inhibitor EK1 are shown in surface and cartoon modes, respectively. (B) Workflow for predicting mutations that may enhance the binding affinity of EK1 to HR1 by mCSM-PPI2. (C) The RMSD values of all complexes during the entire MD simulations. (D-G) The binding free energies between HR1 and the WT/mutant EK1s calculated by MM-PBSA (D and E) or SIE (F and G) method, in which the conformational interval used for calculation is 1 frame (D/F) or different frames (E/G). Dashed lines represent the mean of the calculated results at different intervals. (H) Hierarchical clustering tree for the energy contributions of individual residues. Orange and blue represent favorable and unfavorable energy contributions of residues, and white indicates the corresponding residues have no contribution to the binding. The energy contributions of residues on EK1s of WT, Q1004E, Q1004D, Q1004Y, N1006I, Q1004E/N1006I, Q1004D/N1006I and Q1004Y/N1006I mutants are arranged in order from the inside to the outside, as indicated by the blue arrow.

with higher energy contribution to the binding are mainly distributed in cluster *b*.

Through the above analyses, it can be concluded that the mutant EK1 with dual Q1004E/N1006I mutation is the most competitive inhibitor among WT/mutant EK1s. Therefore, it may be more effective than EK1 in combating SARS-CoV-2. Meanwhile, it is found that twelve residues (L1002, I1005, V1007, F1009, L1010, L1012, L1019, I1023, L1026, Y1030, I1031 and L1033) in cluster *b* are the main contributors of WT/mutant EK1s binding to HR1, which would be helpful to understand their inhibitory mechanisms. We hope that the current study will promote the development of effective inhibitors targeting the conserved HR1 domain of SARS-CoV-2.

Declaration of Competing Interest

All authors claim no conflict of interest.

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References

1. Ye Q., Wang B.L., Mao J.H.. The pathogenesis and treatment of the 'Cytokine Storm' in COVID-19. *J. Infection* 2020;**80**:607–13.
2. Wang Q.H., Zhang Y.F., Wu L.L., Niu S., Song C.L., Zhang Z.Y., et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell* 2020;**181**:894–904.
3. Lan J., Ge J.W., Yu J.F., Shan S.S., Zhou H., Fan S.L., et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 2020;**581**:215–20.
4. Yan F.F., Gao F.. Comparison of the binding characteristics of SARS-CoV and SARS-CoV-2 RBDs to ACE2 at different temperatures by MD simulations. *Brief Bioinform.* 2021;**22**:1122–36.
5. Xia S., Zhu Y., Liu M.Q., Lan Q.S., Xu W., Wu Y.L., et al. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cell Mol. Immunol.* 2020;**17**:765–7.
6. Xia S., Yan L., Xu W., Agrawal Anurodh S., Algaissi A., Tseng Chien-Te K., et al. A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Sci. Adv.* 2019;**5**:eaav4580.
7. Rodrigues C.H.M., Myung Y., Pires D.E.V., Ascher D.B.. mCSM-PPI2: predicting the effects of mutations on protein–protein interactions. *Nucleic Acids Res.* 2019;**47**:W338–44.
8. Chen J.Z., Wang X.Y., Pang L.X., Zhang J.Z.H., Zhu T.. Effect of mutations on binding of ligands to guanine riboswitch probed by free energy perturbation and molecular dynamics simulations. *Nucleic Acids Res.* 2019;**47**:6618–31.
9. Khan A., Zia T., Suleman M., Khan T., Ali S.S., Abbasi A.A., et al. Higher infectivity of the SARS-CoV-2 new variants is associated with K417N/T, E484K, and N501Y mutants: an insight from structural data. *J. Cell Physiol.* 2021;**236**:7045–57.

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COVID-19 persists: Current risk of a recurrence of the epidemic in China

Dear Editor

In December 2019, nearly a month before the traditional Chinese Spring Festival, the first confirmed case of novel coronavirus pneumonia (subsequently named coronavirus disease, COVID-19) appeared in Wuhan, Hubei Province, China, and subsequently spread to other cities with the flow of people during the Spring Festival. On January 20, 2020, after a field investigation in Wuhan, Zhong Nanshan, head of the high-level expert group of the National Health Commission of China (NHCC), confirmed that there was human-to-human transmission of COVID-19 (http://www.xinhuanet.com/politics/2020-01/20/c_1125487200.htm), and subsequently the COVID-19 outbreak quickly became the focus of media worldwide, attracting widespread attention.[1–3] As of December 6, 2021, COVID-19 had spread to about 207 countries with 265,194,191 cases reported worldwide. Some countries have experienced multiple epidemic waves.[4] Despite containment measures taken by national authorities and the international community, the COVID-19 pandemic remains severe. In order to effectively contain the spread of the epidemic, the Chinese government has introduced measures such as lockdown of cities at high risk of COVID-19 epidemics, quarantine, nucleic acid screening, free COVID-19 treatment, mask wearing, regular disinfection, and COVID-19 vaccination.[5] Due to the joint efforts of the Chinese government and the whole nation, the COVID-19 epidemic in China has been gradually contained, with the number of large-area, concentrated outbreaks dropping gradually. However, due to the high number of COVID-19 cases worldwide and the approaching of the traditional Chinese Spring Festival, China still faces a risk of a recurrent COVID-19 epidemic, thereby making vigilance a necessity.

We analyzed the number of confirmed cases and deaths of COVID-19 in China in the past 1 year according to the COVID-19 case data released by the World Health Organization (WHO), and observed that there were two distinct peaks of cases in China in the past year, with the number of weekly confirmed cases being up to 1306 at the first peak and 4051 at the second peak (Fig. 1). According to the data released on the National Health Commission (NHS) website, we analyzed the geographical distribution of confirmed cases in the past 1 year and observed that regions with close international exchange and cooperation, such as Taiwan and Hong Kong, had a relatively high number of confirmed cases (16,011 and 6223, respectively). Moreover, there was a relatively high number of cases in border cities, such as the those in Yunnan(1523), Heilongjiang (1075) and Inner Mongolia (655); as well as in coastal cities, such as Shanghai (1552), Guangdong (1335), Jiangsu (939), and Fujian (836) (Fig. 2), indicating that importation from outside the country is currently the main reason for the increase of cases in China. The number of cases in some densely populated cities such as those in Hebei Province, Sichuan Province, and Henan Province were 1085, 471, and 348, respectively, which were significantly higher than those in less densely populated areas such as those in Xinjiang (1) and Tibet (0) (Fig. 2),

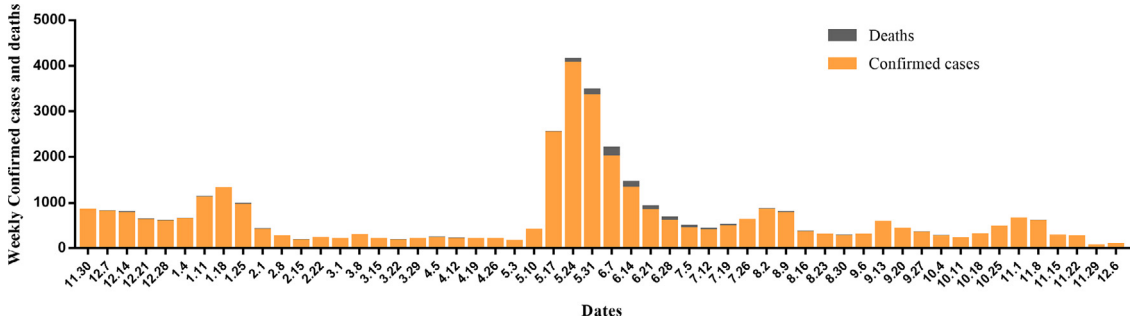

Fig. 1. Number of new confirmed cases and deaths of COVID-19 in China from November 30, 2020 to December 6, 2021. The official statistics for all documented cases of COVID-19 in China were obtained from the WHO (https://covid19.who.int/region/wpro/country/cn).

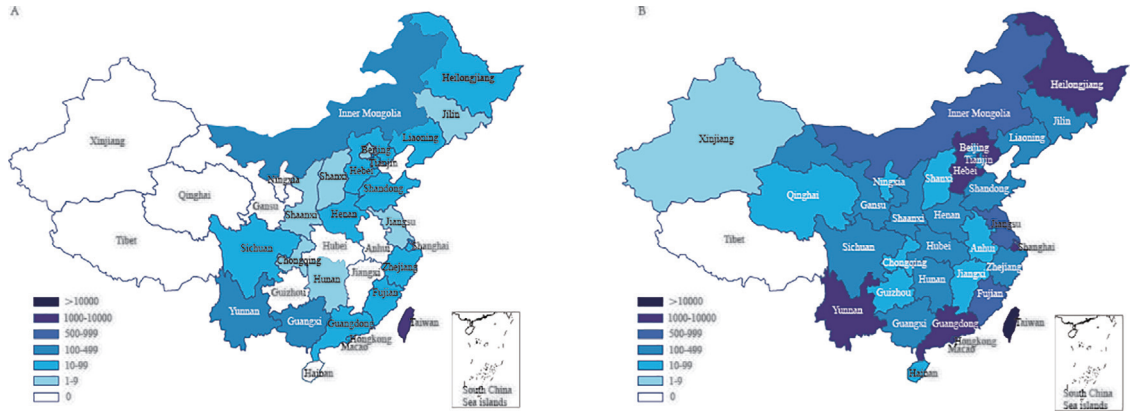

Fig. 2. Geographical distribution of confirmed cases of COVID-19 in China. A. Geographical distribution of current confirmed cases in China as of December 6, 2021; B. Cumulative number of confirmed cases in China from November 30, 2020 to December 6, 2021.The official statistics for all documented cases of COVID-19 throughout China were obtained from the NHS (http://www.nhc.gov.cn/xcs/xxgzbd/gzbd_index.shtml).

suggesting that dense population and high human flow are also important reasons for the COVID-19 outbreaks.

Although the epidemic has been effectively contained in China, the country still faces several risk factors that could cause a recurrence of the epidemic around the time of the Spring Festival: (a) the Spring Festival is a traditional festival in China, and most people reunite with their families during this period, which increases the risk of epidemic spread; (b) the cold climate with low temperatures during the winter increases the risk of COVID-19 infection[6]; (c) the persistence of foreign epidemics and the fact that China is a major importer/exporter country may result in imported infections; (d) the emergence of new mutant strains increases the difficulty of prevention and containment of COVID-19, and the original vaccine may not achieve complete protection against emerging strains,[7] so booster vaccination is needed to maintain the level of immunological protection in the population; (e) China has continued to promote vaccination, and the current vaccination rate has reached approximately 78%, but the majority of the population have not received a booster vaccination does against variant strains and the vaccination rate among children is low; (f) after the long against the COVID-19 epidemic, vigilance among the general public has waned, and mask-wearing and disinfection measures have become less frequently observed. Given the above risk factors, it is clear that care should be taken in China for preventing and containing COVID-19 outbreaks. The government should be more vigilant and should strengthen prevention and containment measures for avoiding new COVID-19 outbreaks, while minimizing economic losses. Additionally, other countries are encouraged to learn from China's example of effective measures for COVID-19 prevention and containment, so as to overcome the COVID-19 pandemic as soon as possible.

Declaration of Competing Interest

None.

Acknowledgments

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References

1. Chan J.F., Yuan S., Kok K.H., To K.K., Chu H., Yang J., et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020;**395**(10223):514–23.
2. Khan T., Agnihotri K., Tripathi A., Mukherjee S., Agnihotri N., Gupta G.. COVID-19: a worldwide, zoonotic, pandemic outbreak. *Altern Ther Health Med* 2020;**26**(S2):56–64.
3. Stuckelberger A., Urbina M.. WHO international health regulations (IHR) vs COVID-19 uncertainty. *Acta Biomed* 2020;**91**(2):113–17.
4. Kwok K.O., Huang Y., Tsoi M.T.F., Tang A., Wong S.Y.S., Wei W.I., et al. Epidemiology, clinical spectrum, viral kinetics and impact of COVID-19 in the Asia-Pacific region. *Respirology* 2021;**26**(4):322–33.
5. Liu N.N., Tan J.C., Li J.. COVID-19 pandemic: experiences in China and implications for its prevention and treatment worldwide. *Curr Cancer Drug Tar* 2020(20):410–16.
6. Li G., Niu J., Fan X.. The chilly climate may increase the chance of infecting COVID-1. *Journal of Infection* 2021(82):e16–17.
7. Milne G., Hames T., Scotton C., Gent N., Johnsen A., Anderson R.M., et al. Does infection with or vaccination against SARS-CoV-2 lead to lasting immunity? *Lancet Respir Med* 2021;**9**(12):1450–66.

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Prediction analysis of porcine AXL protein as a potential receptor for SARS-CoV-2

Dear Editor,

Recent published study reported that angiotensin-converting enzyme 2 (ACE2) of various mammals may contribute to cross-species transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] In fact, there is strong evidence that SARS-CoV-2 is a zoonotic spillover from bats into human populations through bridging hosts.[2] Therefore, ongoing investigation on possible intermediate hosts would facilitate understanding the mechanisms of cross-species transmission of SARS-CoV-2 with potential benefits for the control of the coronavirus disease 2019 (COVID-19).

Notably, the receptor is the crucial host restrict factor in SARS-CoV-2 infection. ACE2 is proved to serve as a primary factor for the cellular entry of SARS-CoV-2, which may determine the host tropism.[3,4] However, the low-abundance expression of ACE2 in human lung and trachea indicated that additional cellular mediators may exist that promote cellular uptake of SARS-CoV-2. Recently research demonstrated that the tyrosine-protein kinase receptor UFO (AXL) is a novel candidate receptor, especially in the respiratory system.[5] Since pigs as important livestock species and the huge size of their global population, it is of great significance to reveal the putative susceptibility of swine to SARS-CoV-2.[6] Although previous data suggested that porcine ACE2 could bind SARS-CoV-2 spike (S),[3] the interaction between porcine AXL and S that predict infection capacity is still unclear. Here, the amino acid (aa) sequences of AXLs in different species containing the residues interfacing with the N-terminal domain (NTD) of S were aligned to screen the critical sites by ESPript 3.0 (https://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi). According the structure of human AXL-S NTD complex, P57–58, E59, H61, I68, E70, E85, F113, and G115 are located in the interface and interact with S NTD. As illustrated in Fig. 1A, the key residues of porcine AXL were consistent with those of mink and ferret, which are efficiently infected with SARS-CoV-2, and showed weaker affinity characteristics than human AXL. Among these sites, T61H and G85E substitutions may reduce the binding affinities, while V68I and E115G mutations would not disrupt the formation of hydrogen bonds.[5] Specifically, G116H substitution existing in hamster, mouse, mink, ferret, and pig AXLs result in a new interaction on the interface, which may have little effect on interfacial binding properties. The distribution of electrostatic potential also confirmed that the more identical pattern in AXL-S interface between pig and ferret, which appears to be significantly different from mouse (Fig. 1B). In consequence, the local structural difference of AXLs among different species suggested that porcine AXL may exhibit the similar binding affinity with S NTD to those of mink and ferret.

Interestingly, the experimental infection results in two independent studies did not support that pig is susceptible to SARS-CoV-2 infection,[7,8] in contrast to previous ACE2 results and the above AXL analysis associated with the spike-binding peculiarity.[1,3] Nevertheless, several porcine cell lines are identified to be permissive for SARS-CoV-2 infection. Owing to the limited pig breeds and numbers, age and health condition should been taken into account in future study,[7] which may combine the pre-existing illnesses to develop typical COVID-19 symptoms.[9] In addition, SARS-CoV-2 possesses the largest RNA genome, and evolves quickly to generate genetic diversity. The newly emerging variants omicron and delta have stronger ACE2 binding affinity and higher contiguous than these predecessors, with the mild clinical manifestations. To address the impact of the certain changes in S NTD of different SARS-CoV-2 strains, comparative analysis of aa sequences was performed. The result in Fig. 2 uncovered that the key residues K147, K150, W152, R246, S247, P251, and S256 in the AXL-S NTD complexes were unaltered, while multiple residue deletions and substitutes occurred in the same positions between two variants. More importantly, whether these aa differences would have an influence on the ability of SARS-CoV-2 to cause COVID-19 related diseases in pigs is urgently needed to be explored. Once the SARS-CoV-2 variants enhance the infectivity and transmission in pigs, the outcome for evolution of zoonotic potential would become more unpredictable and complicated when these variants are introduced into swine herds.

In summary, alignment of AXL from different species and S NTD of three representative SARS-CoV-2 strains would be conducive to quest for the potential recipient hosts in zoonotic transmission. Considering pig as the dominant livestock and the idea human biomedical model,[10] a specific focus on interaction between porcine AXL and S of SARS-CoV-2 variants may also help prevent the risk of next pandemic and food supply stability.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

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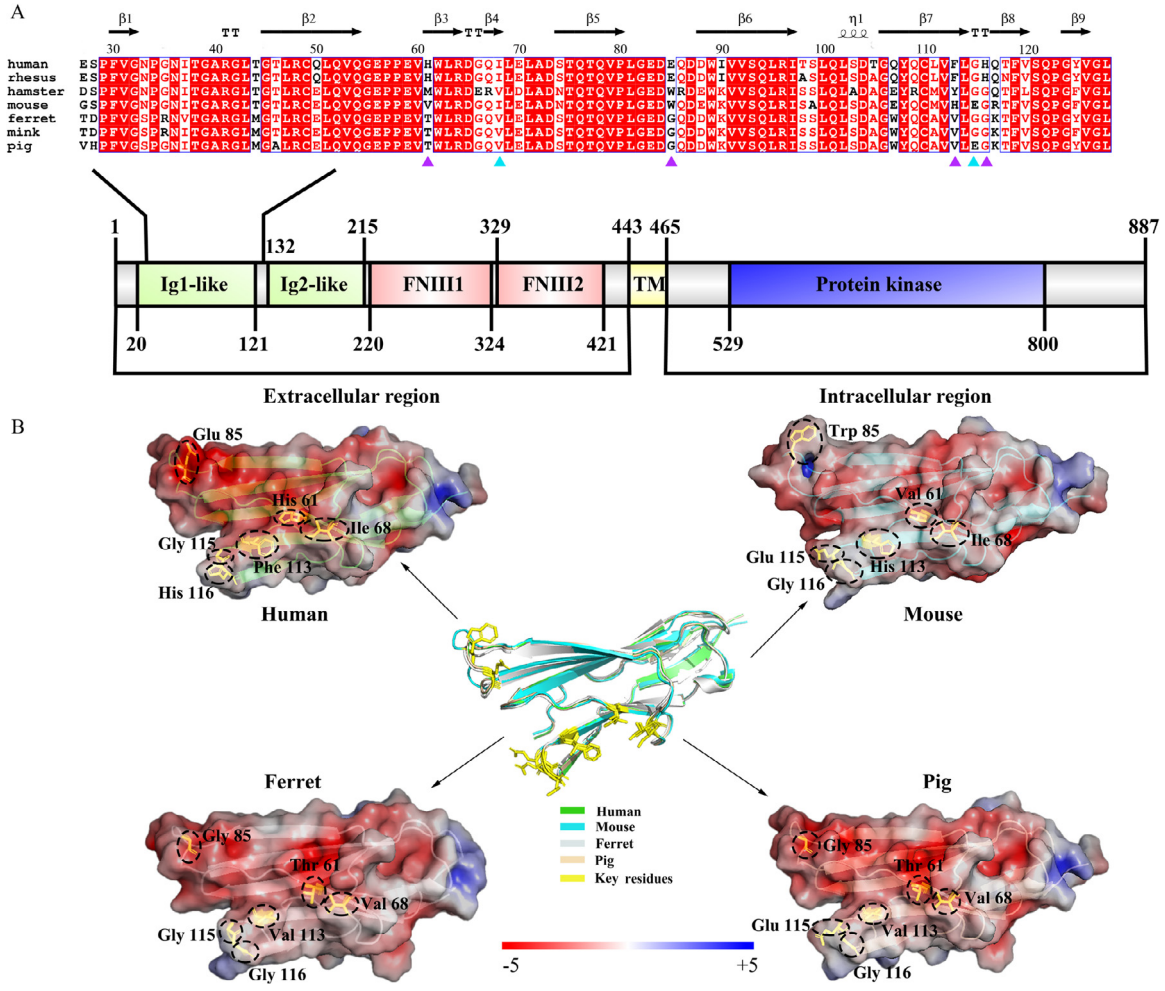

Fig. 1. Alignment and surface potential analysis of crucial amino acids in AXL proteins. (A) Comparative analysis of the residues of AXLs at the interface binding to spike of SARS-CoV-2 from human (GenBank accession no. NP_068713), rhesus (GenBank accession no. EHH30062.1), hamster (GenBank accession no. XP_040590606), mouse (GenBank accession no. NP_033491), ferret (GenBank accession no. XP_004776133), mink (GenBank accession no. XP_044113292.1), and pig (GenBank accession no. NP_001121930). The ALX residues at position 68 and 115 are marked in cyan triangles, position 61, 85, 113, and 116 are labeled with purple triangles. (B) Surface potential diagram of interface zone of AXLs. The structural superposition of the AXL region 27–128 from human (green, PDB code 4RA0), mouse (cyan, Uniprot entry Q0093), ferret (gray), and pig (wheat) is in the center. The AXL structures of pig and ferret were modeled using the homology models of human AXL (PDB code 4RA0 and 5VXZ, respectively) as the templates by SWISS-MODEL (https://swissmodel.expasy.org/). The six key differential residues of AXL interacting with spike protein of SARS-CoV-2 are represented by yellow sticks in the structural superposition, the details of which are further circled by dash line in the surface electrostatic potential maps of each AXL. The electrostatic potential color range is -/+5.

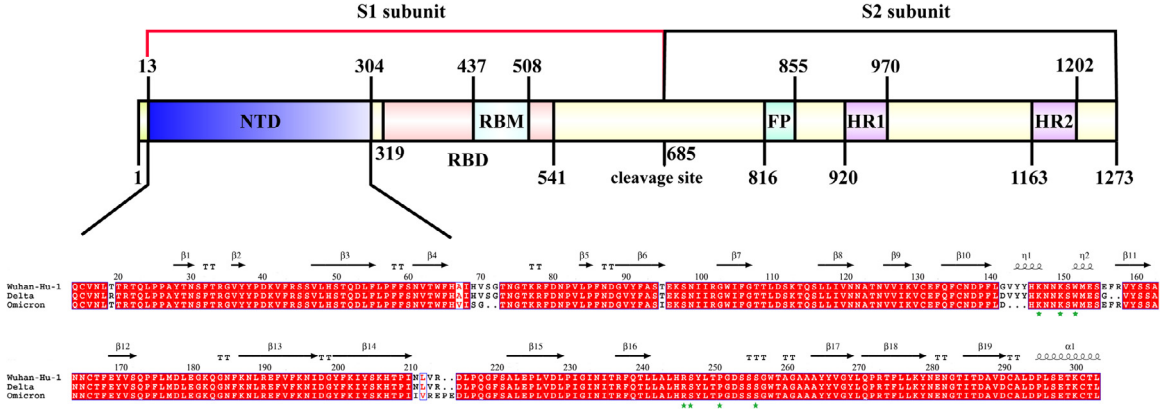

Fig. 2. Sequence alignment of spike protein from three SARS-CoV-2 strains including Wuhan-Hu-1 (GenBank accession no.YP_009724390), delta variant (GenBank accession no. QYM88683), and omicron variant (GenBank accession no. UFS23237). The important residues in NTD of S interacting with AXL are marked with green stars.

data collection and interpretation, or the decision to submit the work for publication.

References

1. Li R., Qiao S., Zhang G.. Analysis of angiotensin-converting enzyme 2 (ACE2) from different species sheds some light on cross-species receptor usage of a novel coronavirus 2019-nCoV. *J Infect* 2020;**80**(4):469–96 PubMed PMID: 32092392.
2. Ruiz-Aravena M., McKee C., Gamble A., Lunn T., Morris A., Snedden C.E., et al. Ecology, evolution and spillover of coronaviruses from bats. *Nat Rev Microbiol* 2021:1–16. PubMed PMID: 34799704 https://www.nature.com/articles/s41579-021-00652-2#Sec2.
3. Zhou P., Yang X.L., Wang X.G., Hu B., Zhang L., Zhang W., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;**579**(7798):270–3 PubMed PMID: 32015507.
4. Ren W., Zhu Y., Wang Y., Shi H., Yu Y., Hu G., et al. Comparative analysis reveals the species-specific genetic determinants of ACE_2 required for SARS-CoV-2 entry. *PLoS Pathog* 2021;**17**(3):e1009392 PubMed PMID: 33760889.
5. Wang S., Qiu Z., Hou Y., Deng X., Xu W., Zheng T., et al. AXL is a candidate receptor for SARS-CoV-2 that promotes infection of pulmonary and bronchial epithelial cells. *Cell Res* 2021;**31**(2):126–40 PubMed PMID: 33420426.
6. Muñoz-Fontela C., Dowling W.E., Funnell S.G.P., Gsell P.S., Riveros-Balta A.X., Albrecht R.A., et al. Animal models for COVID-19. *Nature* 2020;**586**(7830):509–15 PubMed PMID: 32967005.
7. Schlottau K., Rissmann M., Graaf A., Schön J., Sehl J., Wylezich C., et al. SARS–CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *Lancet Microbe* 2020;**1**(5):e218–ee25 PubMed PMID: 32838346.
8. Shi J., Wen Z., Zhong G., Yang H., Wang C., Huang B., et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science* 2020;**368**(6494):1016–20 PubMed PMID: 32269068.
9. Du X., Guo Z., Fan W., Hai T., Gao F., Li P., et al. Establishment of a humanized swine model for COVID-19. *Cell Discov* 2021;**7**(1):70 PubMed PMID: 34404772.
10. Lunney J.K., Van Goor A., Walker K.E., Hailstock T., Franklin J., Dai C. Importance of the pig as a human biomedical model. *Sci Transl Med* 2021;**13**(621):eabd5758 PubMed PMID: 34818055.

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Increased mortality in COVID-19 patients with fungal co- and secondary infections admitted to intensive care or high dependency units in NHS hospitals in England

Dear Editor,

We read with interest the recent systematic review and meta-analysis by Lansbury et al. which aimed to evaluate the burden of bacterial and fungal co-infections in patients with COVID-19, highlighting the challenges in diagnosing certain fungal infections in this setting and the need for a high level of suspicion of fungal infections in critically-ill COVID-19 patients.[1] Severe COVID-19 disease does predispose patients to fungal co- and particularly secondary infections, with reports describing COVID-associated pulmonary aspergillosis (CAPA) and invasive yeast infections (particularly candidaemia) predominating.[2–10] Additionally, a devastating wave of COVID-associated mucoromycosis (CAM) impacted the Indian subcontinent in late spring, with tens of thousands of cases reported in India alone.[11] Anecdotal reports and individual case series suggested that each of these fungal complications of COVID-19 increases the risk of poor outcomes, including increased hospital lengths of stay (LoS) and excess mortality.[4,7,8,10] A recent French multi-center study confirmed significant increased mortality in patients with possible or probable CAPA.[12] Here we report data to quantify the impact of fungal co- and secondary infections on outcomes of patients admitted to intensive care units (ICU) in NHS hospitals in England with laboratory confirmed COVID-19 and likely or proven fungal disease, concentrating on CAPA and COVID-associated candidaemia (CAC), since few UK cases of CAM have been reported as of November 2021.

De-duplicated data on patients with candidaemia or aspergillosis (March 2020-March 2021) were collated from the UK Health Security Agency (UKHSA) UK National Mycology Reference Laboratory (MRL); candidaemia patients were also collated from the Communicable Disease Reporting module of UKHSA's Second Generation Surveillance System. Laboratory confirmed cases of COVID-19 were retrieved from the PHE National Incident Coordination center Epidemiology Cell (NICCEC). Data on Intensive Care Unit (ICU) admissions in NHS hospitals in England was collected from Secondary Uses Service (SUS) +, NHS Digital, and sorted into total ICU stays, before being linked to the laboratory confirmed COVID-19 cases to create a dataset of patients admitted to ICU with COVID-19.

Candida data was first linked to the NICCEC data to determine which of the candidemia patients also had a COVID-19 diagnosis. Patients with diagnoses of candidemia that were more than 10 days before or 60 days after a COVID-19 diagnosis were excluded, except where the patients had remained hospitalised for the entire period between diagnoses. Retained records were then linked to the ICU dataset of COVID-19 admissions. Data linkage across the datasets was performed in 3 stages to capture patients with missing key identifiers using: (i) NHS number and date of birth, (ii) date of birth and soundex of both surname and forename, (iii) NHS number and soundex of both surname and forename. After data linkage and exclusion, 34,550 patient records were available for analysis, 271 of which highlighted patients with CAC. Remaining patients with COVID-19 with no evidence of either *Aspergillus* or candidaemia co/secondary infection formed the reference group. CAC cases were categorised as (i) preceding COVID-19 diagnosis ($N = 6$; recovery of *Candida* sp. from blood between 10 and 1 days pre-COVID-19 test positivity); (ii) co-infection ($N = 7$; blood culture positivity within 1 day either side of COVID-19 test positivity) and (iii) secondary infection in 258 patients (blood culture positivity 2 to 60 days post COVID-19 diagnosis in 255 patients, plus 3 cases of candidaemia >60 days post COVID-19 diagnosis in patients who had remained hospitalised for the entire duration). Among the ICU cohort, 60-day crude case fatality rate (CFR) for patients

with CAC was 57.2% (95% CI 48.5–66.9; 155/271 patients), compared to 39.9% (95% CI 39.3–40.6; 13,684/34,279 patients) for patients with COVID-19 alone ($p < 0.001$). When adjusted for age and sex, both CFRs decreased, to 37.5% (95% CI 25.8–50.7) in patients with CAC and 25.5% (95% CI 24.8–26.2) in patients with COVID-19 alone ($p = 0.058$). Finally, median LoS in ICU patients with CAC was substantially longer (25 days; IQR 14–43; $N = 270$) than median LoS among ICU patients with COVID-19 alone (7 days; IQR 3–16; $N = 34{,}057$; $p < 0•001$). Median time from ICU admission to the *Candida* sample was 14 days (IQR 8–21), with patients then staying in ICU for a median of 10 days (IQR 2–27) from the fungal diagnosis to discharge.

For CAPA, accurate evaluation of incidence is confounded by multiple factors, including lower likelihood of bronchoalveolar testing of severely ill COVID-19 patients in ICU settings (due to risk of transmission from aerosols), geographic differences in biomarker testing and reporting (which influence incidence estimates), lack of a universally employed diagnostic algorithm and the fact that recovery of an *Aspergillus* spp. isolate from a COVID-19 respiratory sample is not proof of invasive disease.[2,3,8] Given these constraints, a different approach to estimating excess mortality was employed. Initial data linkage and exclusion was performed against the NICCEC and SUS+ ICU data as above, starting with all UK patients from whom an isolate of *Aspergillus fumigatus* had been referred to the MRL from any respiratory secretion (sputum, tracheal aspirate, directed and non-directed bronchial lavages) from March 2020-March 2021. After linkage 34,398 patients with COVID-19 were available, of which 119 had *Aspergillus fumigatus* isolated from a respiratory specimen. For these 119 patients, the MRL Information Management System was interrogated for fungal biomarker testing (serum galactomannan [GM] or (1–3)-β-d-glucan, respiratory sample GM, *Aspergillus*-specific PCR) that could contribute towards a diagnosis of CAPA. A resulting 28 patients were stratified as possible/probable CAPA (multiple positive biomarker tests), 22 patients as unlikely to have CAPA (biomarker tests all negative), and 69 patients for whom biomarker tests were unavailable (unknown CAPA).[9] The crude CFR for COVID-19 cases admitted to ICU with an *Aspergillus fumigatus* sample was 54.6% (95% CI 42.2–69.9; 65/119 patients). Adjusted for age and sex, this reduced to 44.4% (95% CI 25.6–66.9), higher than the COVID-19 ICU patients with no fungal infection (25.5%; $p = 0.073$). Looking to mortality by CAPA classification, crude CFRs were 67.9% (95% CI 40.8–100.0; 19/28) for possible/probable CAPA and 50.5% (95% CI 37.0–67.4; 46/91) for unlikely or unknown CAPA combined. Numbers were too small to look at age and sex adjusted CFRs by these categories. Finally, median LoS was significantly longer in possible/probable CAPA patients (29.5 days, IQR 23.5–43.5, $n = 28$) than in matched COVID-19 patients in ICU (median LoS 7 days; IQR 3–16; $n = 34{,}057$; $p < 0.001$). Median time from ICU admission to the *Aspergillus* sample was similar between possible/probable CAPA patients (16.5 days, IQR 11.5–26.5) and unknown/unlikely CAPA patients (17 days, IQR 12–24). This was also the case for time to discharge after the fungal sample, with the median time being 6.5 days (IQR 1.5–20) and 6 days (IQR −1–21) respectively.

This analysis has limitations. Cases of candidemia and CAPA are likely to be significantly underestimated as they relied upon laboratories reporting and/or referring isolates to the MRL. Laboratories may have undertaken in-house biomarker testing to aid in the diagnosis of CAPA, which would have helped to better stratify the patients in the "unknown CAPA" cohort. Linkage to SUS+ ICU data could only be done by NHS number and date of birth, so any COVID-19 patients missing these data could not be included. Nonetheless, these estimations for excess mortality caused by the two most common fungal co-/secondary infections encountered with severe COVID-19 align with prior smaller reports from individual centers (nationwide and worldwide) and the recent French study on CAPA in that mortality and LoS are increased in patients with COVID-19 and fungal co-/secondary infection.[4,7,10,12] This data analysis further emphasises the importance of raised awareness, testing, rapid diagnosis, surveillance and prompt and appropriate treatment of fungal infections in severely ill patients with COVID-19.

Declaration of Competing Interest

The authors have no competing interests to declare.

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References

1. Lansbury L., Lim B., Baskaran V., Lim W.S.. Co-infections in people with COVID-19: a systematic review and meta-analysis. *J Infect* 2020;**81**:266–75.
2. Arastehfar A., Carvalho A., van de Veerdonk F.L., et al. COVID-19 associated pulmonary aspergillosis (CAPA)-from immunology to treatment. *J Fungi* 2020;**6**:E91. doi:10.3390/jof6020091.
3. Verweij P.E., Gangneux J.P., Bassetti M., et al. Diagnosing COVID-19-associated pulmonary aspergillosis. *Lancet Microbe* 2020. doi:10.1016/S2666-5247(20)30027-6.
4. White P.L., Dhillon R., Cordey A., et al. A national strategy to diagnose COVID-19 associated invasive fungal disease in the ICU. *Clin Infect Dis.* 2020 ciaa1298. doi:10.1093/cid/ciaa1298.
5. Borman A.M., Palmer M.D., Fraser M., et al. COVID-19-associated invasive aspergillosis: data from the UK national mycology reference laboratory. *J Clin Microbiol* 2020;**59**(1) e02136-20doi. doi:10.1128/JCM.02136-20.
6. Armstrong-James D., Youngs J., Bicanic T., et al. Confronting and mitigating the risk of COVID-19. *Eur Respir J* 2020;**56**(4):2002554 doi. doi:10.1183/13993003.02554-2020.
7. Bartoletti M., Pascale R., Cricca M., et al. Epidemiology of invasive pulmonary aspergillosis among COVID-19 intubated patients: a prospective study. *Clin Infect Dis* 2020:ciaa1065. doi:10.1093/cid/ciaa1065.
8. Salmanton-García J., Sprute R., Stemler J., et al. COVID-19-associated pulmonary aspergillosis, March-August 2020. *Emerg Infect Dis* 2021;**27**:1077–86. doi:10.3201/eid2704.204895.
9. Koehler P., Bassetti M., Chakrabarti A., et al. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. *Lancet Infect Dis* 2021;**21**:e149–62. doi:10.1016/S1473-3099(20)30847-1.
10. Riche C.V.W., Cassol R., Pasqualotto A.C.. Is the frequency of candidemia increasing in COVID-19 patients receiving corticosteroids? *J Fungi* 2020;**6**(4):286 Basel13PMID: 33203016; PMCID: PMC7712895. doi:10.3390/jof6040286.
11. Raut A., Huy N.T.. Rising incidence of mucormycosis in patients with COVID-19: another challenge for India amidst the second wave? *Lancet Respir Med* 2021;**S2213**(21) -260000265-4. doi:10.1016/S2213-2600(21)00265-4.
12. Gangneux J.P., Dannaoui E., Fekkar A., et al. Fungal infections in mechanically ventilated patients with COVID-19 during the first wave: the French multicentre MYCOVID study. *Lancet Respir Med* 2021 S2213-2600(21)00442-2. doi:10.1016/S2213-2600(21)00442-2.

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Necrotizing cellulitis caused by Kodamaea ohmeri fungemia in a HIV- infected patient

Dear Editor,

Kodamaea (Pichia) ohmeri was formerly considered a contaminant, but is now known to be a significant human pathogen that has been shown to cause fungemia, endocarditis, cellulitis, and peritonitis in immunocompromised patients.[1–4]

K. *ohmeri* is a yeast is frequently mistaken for Candida, which belongs to the same family.[1–3] We present this case of fungemia caused by fluconazole-resistant *K. ohmeri* in HIV-infected patient with persistent tinea pedis and severe cellulitis complicating necrotizing fascitis.

A 52-year-old male was diagnosed as HIV infection with antiretroviral therapy (ART) for 5 years but had poor compliance. The CD4 count revealed 132/dL at admission. He had right lower foot severe cellulitis and tinea pedis complicating necrotizing fascitis (Fig. 1A). The patient was sent to the emergency room due to fever, leg edema, redness, and progressive pain. During hospitalization, he received central venous catheter insertion for fluid supply, antibiotic therapy and surgical debridement (Fig. 1A). Series of examinations including blood cultures and wound culture were performed. On hospital day 7, the wound and blood culture yielded a yeast colony (Fig. 1B and C). Fluconazole was given empirically, but had no effect. This yeast-like microorganism was identified as *K. ohmeri* 2 days later. The score value of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) and the Bruker Biotyper of *K. ohmeri* showed 2.1, respectively. Antifungal susceptibility testing was performed with the ATB-Fungus system, and a high minimum inhibitory concentration (level up to 64 mg/l) for fluconazole was found. Fluconazole was replaced with amphotericin B deoxylate. The fever and cellulitis inflammation gradually subsided after surgical debridement and anti-fungal therapy. The patient was discharged in a stable condition.

Kodamaea (Pichia) ohmeri is an unusual pathogen of mycosis.[1–4] In the last 20 years, only 20 cases have been described in the literature.[1–4,8,9] All reported cases had significant predisposing con-

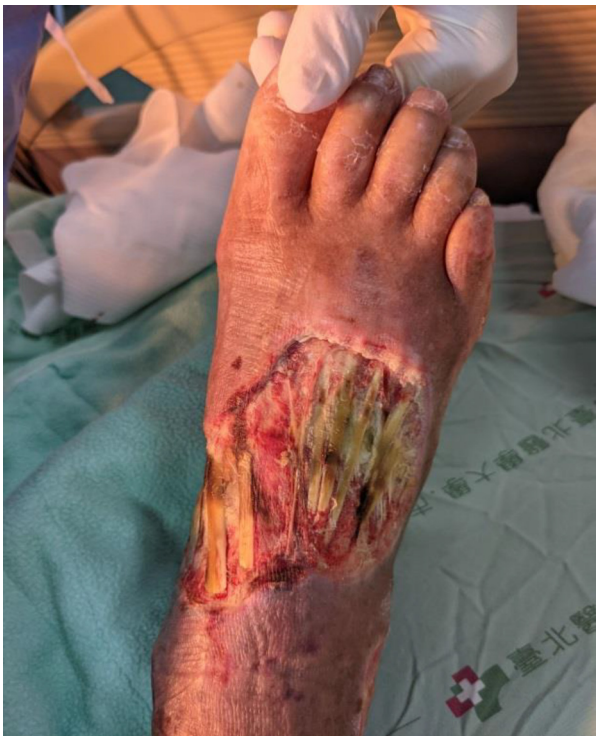

Fig. 1. A The HIV-infected patient had severe cellulitis complicating necrotizing fasciitis and received surgical debridement during hospitalization. **B.** The morphology of *Kodamaea ohmeri* by gram stain (microscopy 1000X) showed pigmented yeast. **C.** The colony of *Kodamaea ohmeri* showed gray-whitish color on the Sabouraud Dextrose agar (SDA).

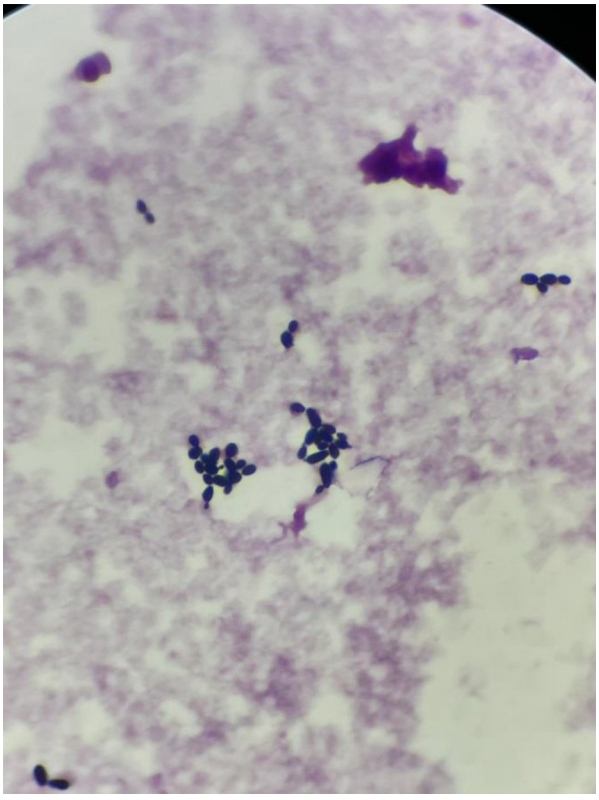

Fig. 1. Continued

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ditions including diabetes mellitus, malignancy, use of catheters, etc.[1–9] However, the association between cellulitis of tinea pedis and fluconazole-resistant *K. ohmeri* in HIV-infected patient has not been described.[1,2] The first clinical isolate was from a pleural effusion and was considered a contaminant.[2] Twenty cases of *K. ohmeri* infection, including the one in this study, have been reported.[1–4,8,9] Among them, 17 were fungemia, and the remaining three were peritonitis, funguria, and polymicrobial wound infection[1–4]. Our patient developed *K. ohmeri* fungemia and was the second reported case with severe cellulitis.[1]The symptoms of *K. ohmeri* infection varied, and included fever, acute pain, and malaise. All the patients were immunocompromised.[1,2,8,9] Seventy percent of *K. ohmeri* and invasive non-albican candidiasis were catheter-related,[4–9] suggesting that breakdown of the skin mucosal barrier is a significant risk factor for invasive fungal infection. Our patient had significant tinea infection and cellulitis of the lower extremities and in immunocompromised state (CD4: 132/dL), which could have contributed to this episode of fungemia.

The current treatment strategy for *K. ohmeri* infections includes the removal of medical devices (e.g., Foley catheter, central venous catheter, and pacemaker) and the use of effective antifungal agents.[1–3,8,9] Previous reports have suggested that amphotericin B and fluconazole are effective in the treatment of *K. ohmeri* fungemia.[1–3] However, there is no definite indication as to which drug is superior to the others.[8,9] Combined treatment with antifungal agents, surgical debridement, and removal of an implanted device or catheter in previous cases [1–3,8,9] has contributed to better outcomes than medical therapy alone. However, 40% of all cases died despite antifungal therapy.[1–3,6–9] Therefore, *K. ohmeri* infection in immunocompromised patients should be considered a potentially critical condition.

In conclusion, the evidence indicates that *K. ohmeri* should be added to the list of potential yeast pathogens that can cause systemic infections in humans of all ages, particularly in immunocompromised patients.[1–3,8,9] Because increasing numbers of cases of *K. ohmeri* fungemia have been reported with high mortality, early diagnosis and appropriate treatment with surgical debridement are crucial in the management of this potentially fatal infection.

Ethics approval

Ethics approval was not required for this study.

Declaration of Competing Interest

The authors have no competing interests to declare.

References

1. Yang B.H., Peng M.Y., Hou S.J., Sun J.R., Lee S.Y., Lu J.J.. Fluconazole-resistant Kodamaea ohmeri fungemia associated with cellulitis: case report and review of the literature. *Int J Infect Dis* 2009;**13**:e493–7 Epub 2009 May 2. PMID: 19411182. doi:10.1016/j.ijid.2009.02.003.
2. Han X.Y., Tarrand J.J., Escudero E.. Infections by the yeast Kodomaea (Pichia) ohmeri: two cases and literature review. *Eur J Clin Microbiol Infect Dis* 2004;**23**:127–30 Epub 2004 Jan 14. PMID: 14722784. doi:10.1007/s10096-003-1067-3.
3. Chi H.Y., Su Y.S., Chen F.L., Lee W.S., Wang C.H.. Breakthrough fungemia caused by Yarrowia lipolytica in a patient with gastric adenocarcinoma during echinocandin therapy. *J Infect* 2021;**82**:e52–3 Epub 2020 Dec 16. PMID: 33338504. doi:10.1016/j.jinf.2020.12.008.
4. Chen Y., Zhao J., Han L., Qi L., Fan W., Liu J., et al. Emergency of fungemia cases caused by fluconazole-resistant Candida auris in Beijing, China. *J Infect* 2018;**77**:561–71 Epub 2018 Sep 13. PMID: 30219662. doi:10.1016/j.jinf.2018.09.002.
5. Lai C.C., Lee M.R., Hsiao C.H., Tan C.K., Lin S.H., Liao C.H., et al. Infections caused by Candida lipolytica. *J Infect* 2012;**65**:372–4.
6. Chitasombat M.N., Kofteridis D.P., Kiang Y.T., Tarrand J., Lewis R.E., Kontoyiannis D.P.. Rare opportunistic (non-Candida, non-Cryptococcus) yeast bloodstream infections in patients with cancer. *J Infect* 2012;**64**:68–75.
7. Wang C.H., Hsueh P.R., Chen F.L., Lee W.S.. Breakthrough fungemia caused by Rhodotorula mucilaginosa during anidulafungin therapy. *J Microbiol Immunol Infect* 2019;**52**:674–5.
8. Shang S.T., Lin J.C., Ho S.J., Yang Y.S., Chang F.Y., Wang N.C.. The emerging life-threatening opportunistic fungal pathogen Kodamaea ohmeri: optimal treatment and literature review. *J Microbiol Immunol Infect* 2010;**43**:200–6.
9. Diallo K., Lefevre B., Cadelis G., Gallois J.C., Gandon F., Nicolas M., et al. A case report of fungemia due to Kodamaea ohmeri. *BMC Infect Dis* 2019;**19**:570. doi:10.1186/s12879-019-4208-8.

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Incident changes in the prevalence of respiratory virus among children during COVID-19 pandemic in Hangzhou, China

Dear Editor,

The report entitled "Nonpharmaceutical interventions reduced the incidence and exacerbation of allergic diseases in children during the COVID-19 pandemic" by Ye et al. aroused our strong concern.[1] In a report, Ye et al. announced that the incidence of allergic diseases in children during the COVID-19 epidemic was reduced and that the exacerbation of diseases in allergic patients was also reduced. Here, we presented the prevalence of the respiratory virus among children during the COVID-19 pandemic in Hangzhou, China.

Adenoviruses (Ads) are nonenveloped icosahedral viruses with a diameter of ∼ 90 nm and slight structural differences between genotypes. More than 80% of diagnosed adenovirus infections occur in children under four years old (due to lack of humoral immunity).[2] Adenovirus infections may occur in healthy children or adults in closed or crowded environments (particularly military recruits).[2,3]

Respiratory syncytial virus (RSV) was discovered more than 50 years ago. It has since been identified as the most common cause of acute respiratory tract infections in infants.[4–6] Influenza viruses are members of the Orthomyxoviridae family. There are seven genera: Alpha influenza virus (influenza A virus), Beta influenza virus (influenza B virus), Gamma influenza virus (influenza C virus), Delta influenza virus (influenza D virus), Isa virus, Quaranja virus and Thogoto virus.[7] Influenza A and B viruses often cocirculate annually during seasonal epidemics and can cause severe respiratory diseases in humans. The clinical course of both influenza A and B virus infections can vary from mild symptoms in some cases to a severe respiratory infection characterized by clinical complications that lead to hospitalization and death in some cases.[8]

Infection with these respiratory viruses has a certain seasonality. According to clinical observations, during the COVID-19 pandemic in Hangzhou, there was a significant difference in the number of children with respiratory infections.

The Health Commission of Zhejiang Province released confirmed cases of COVID-19 in Zhejiang Province on January 23, 2020. From the end of 2020 to the beginning of 2021, vaccinations against SARS-CoV-2 for all age groups were launched one after another. In this study, we analyzed children who came to the outpatient clinic of Children's Hospital of Zhejiang University School of Medicine from January 2019 to October 2021, analyzed the number of positive people for the above four respiratory viruses (Fig. 1), and calculated the positive detection rate (number of positive detections of a certain virus/number of visits in the same period) (Fig. 2). Therefore, in this study, we compared the number of respiratory virus infections and positive infection rates in children during the year before the COVID-19 pandemic, the first year after the outbreak, and the second year after the outbreak (mass vaccination) to explore the prevalence of the respiratory virus in the COVID-19 pandemic.

According to the data results, in 2020, there were the fewest outpatient visits and the fewest positive infections in the three years, followed by 2021. The number of visits in 2019 was the largest. In 2019, adenoviruses, influenza A and influenza B had apparent seasonality, while their seasonality was not fully highlighted in 2020 and 2021. The number of infections and infection rates was highest in 2019, followed by 2021, and the lowest in 2020. The number in 2021 is higher than that in 2020, which may be related to the short-term relaxation of protection awareness after universal vaccination. The respiratory syncytial virus showed a rebound increase in 2021 (higher than 2019). Data for 2021 show that people's awareness of protection may decrease slightly after vaccination, resulting in more infections than in 2020. On the other hand, wearing a mask for a long time may increase children's susceptibility to respiratory viruses, which requires further research.

To simplify, the COVID-19 pandemic has changed the prevalence of respiratory viruses among children in Hangzhou. This change may not be due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) itself but mainly due to a series of strict measures taken during the COVID-19 pandemic. First, strict control measures, such as wearing masks and prohibiting large-scale gatherings, have cut off the transmission of respiratory viruses to a certain extent. At the same time, improving people's awareness of protection and the importance of physical fitness has also reduced the infection of respiratory viruses. China has adopted a "dynamic zero" policy against SARS-CoV-2. The spread of SARS-CoV-2 in China is currently under control, but the global pandemic continues, and new variants of SARS-CoV-2 have emerged.[9,10] Therefore, the prevalence of respiratory viruses in children is still uncertain and deserves our attention and continuous monitoring. It should be noted that the limitations of this study are as follows: as a single-center study, conclusions in other regions may be different. The specific measures controlling the infection of respiratory viruses should be further researched in multiple centers.

In short, during the COVID-19 pandemic, nonpharmacological interventions reduced the infection rate of children's respiratory viruses. Continuous testing helps prevent a major outbreak of respiratory virus infection in the later stages of the epidemic.

Declaration of Competing Interest

All authors have declared that there are no conflicts of interest.

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References

1. Ye Q., Liu H., Shang S.. Non-pharmaceutical interventions reduced the incidence and exacerbation of allergic diseases in children during the COVID-19 pandemic. *J Infect* 2021 Oct 30. PubMed PMID: 34728266. Epub 2021/11/04. eng.
2. Chang S.Y., Lee C.N., Lin P.H., Huang H.H., Chang L.Y., Ko W., et al. A community-derived outbreak of adenovirus type 3 in children in Taiwan between 2004 and 2005. *J Med Virol* 2008;**80**(1):102–12 JanPubMed PMID: 18041026. Epub 2007/11/28. eng.
3. Sanchez J.L., Binn L.N., Innis B.L., Reynolds R.D., Lee T., Mitchell-Raymundo F., et al. Epidemic of adenovirus-induced respiratory illness among US military recruits: epidemiologic and immunologic risk factors in healthy, young adults. *J Med Virol* 2001;**65**(4):710–18 DecPubMed PMID: 11745936. Epub 2001/12/18. eng.
4. Ye Q., Liu H.. Impact of non-pharmaceutical interventions during the COVID-19 pandemic on common childhood respiratory viruses - a epidemiological study based on hospital data. *Microbes Infect* 2021;**24**(1):104911 Dec 3PubMed PMID: 34871774. Pubmed Central PMCID: PMC8641407. Epub 2021/12/07. eng.
5. Ye Q., Fu J.F., Mao J.H., Shang S.Q.. Haze is a risk factor contributing to the rapid spread of respiratory syncytial virus in children. *Environ Sci Pollut Res Int* 2016;**23**(20):20178–85 OctPubMed PMID: 27439752. Epub 2016/07/22. eng.
6. Tian D.D., Jiang R., Chen X.J., Ye Q.. Meteorological factors on the incidence of MP and RSV pneumonia in children. *PLoS One* 2017;**12**(3):e0173409 PubMed PMID: 28282391. Pubmed Central PMCID: PMC5345804. Epub 2017/03/11. eng.
7. Asha K., Kumar B.. Emerging influenza D virus threat: what we know so far!. *J Clin Med* 2019;**8**(2):192 Feb 5PubMed PMID: 30764577. Pubmed Central PMCID: PMC6406440. Epub 2019/02/16. eng.
8. Krammer F., Smith G.J.D., Fouchier R.A.M., Peiris M., Kedzierska K., Doherty P.C., et al. Influenza. *Nat Rev Dis Prim* 2018;**4**(1):3 Jun 28PubMed PMID: 29955068. Pubmed Central PMCID: PMC7097467. Epub 2018/06/30. eng.
9. Han X., Ye Q.. The variants of SARS-CoV-2 and the challenges of vaccines. *J Med Virol* 2021 Dec 9. PubMed PMID: 34890492. Epub 2021/12/11. eng.

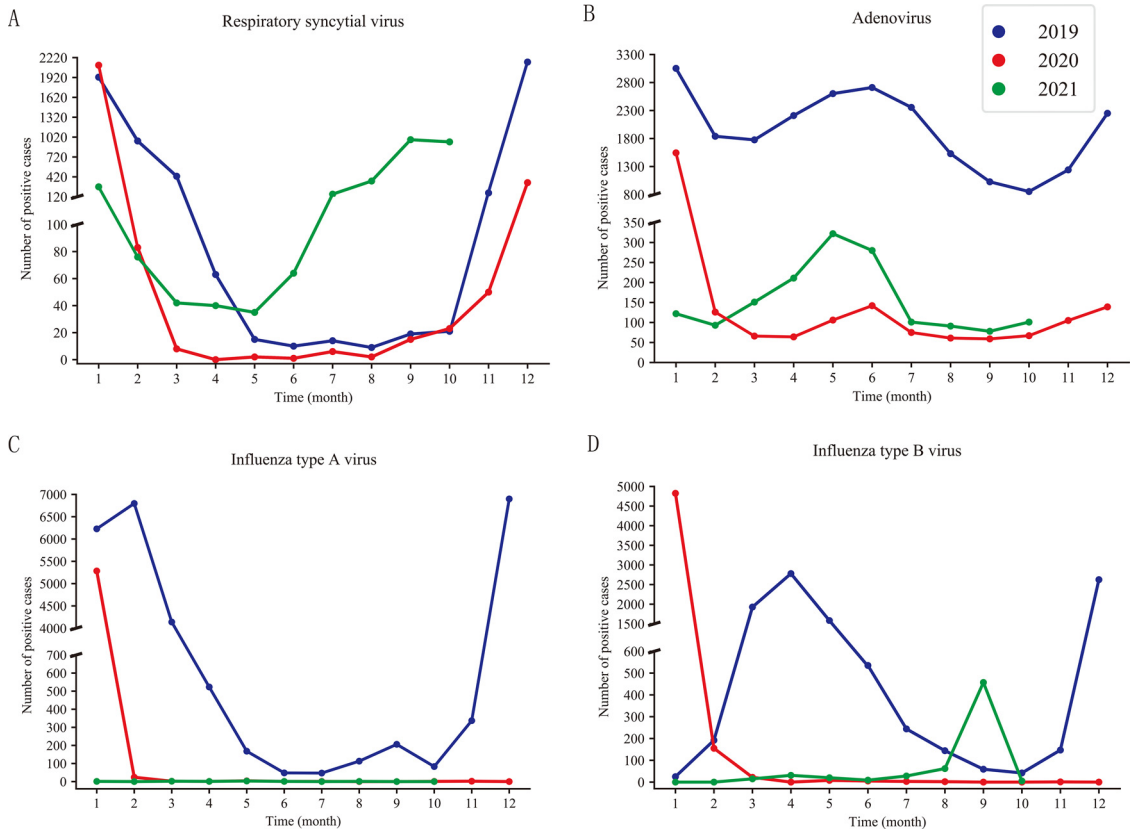

Fig. 1. The number of positive detections of various respiratory viruses at different times (years).

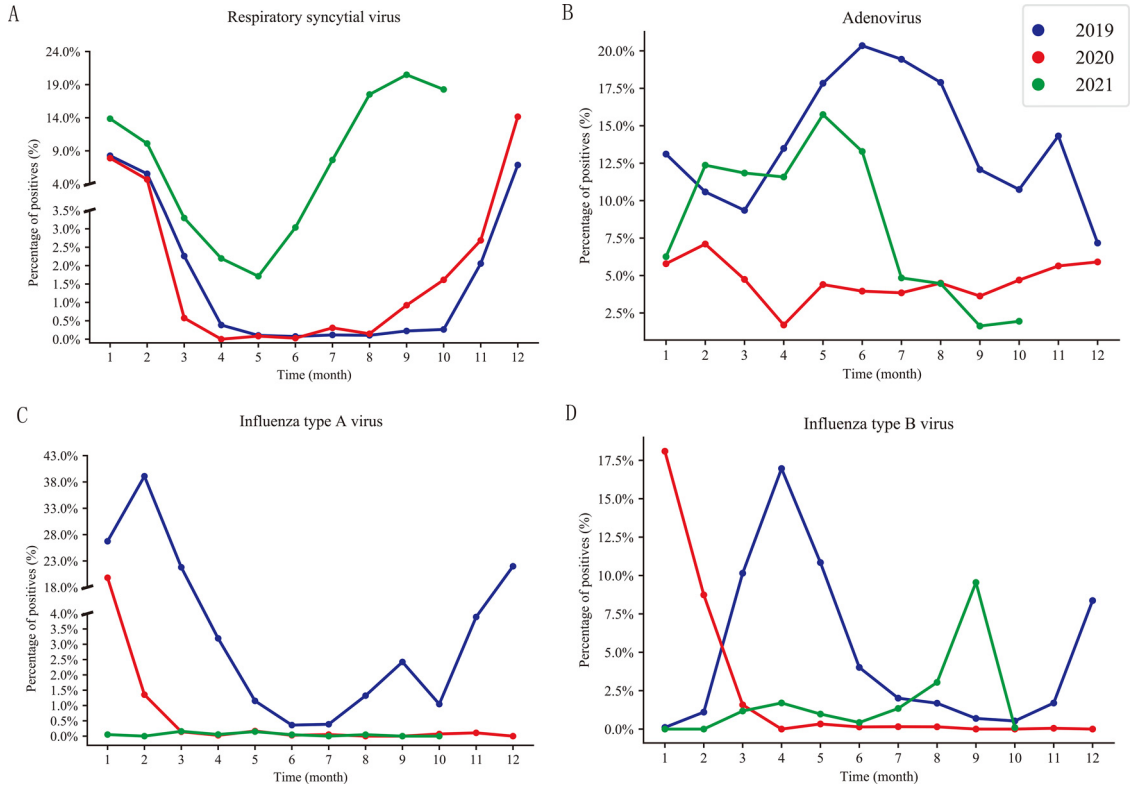

Fig. 2. The positive detection rate of various viruses at different times (years).

10. Tian D., Sun Y., Zhou J., Ye Q.. The global epidemic of the SARS-CoV-2 delta variant, key spike mutations and immune escape. *Front Immunol* 2021;**12**:751778 PubMed PMID: 34917076. Pubmed Central PMCID: PMC8669155. Epub 2021/12/18. eng.

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Impact of time elapsed since full vaccination on SARS-CoV-2 RNA load in Delta-variant breakthrough COVID-19

Dear Editor,

We recently commented on the study by Teyssou and colleagues[1] showing substantial differences across SARS-CoV-2 variants of concern RNA loads in the upper respiratory tract (URT). Regarding the Delta variant, we reported a trend towards higher viral loads in non-vaccinated individuals with COVID-19 compared to those vaccinated with a variety of COVID-19 vaccines.[2] A number of studies concur on that although SARS-CoV-2 RNA loads in the upper respiratory tract of individuals with Delta variant breakthrough infection are comparable to those found in unvaccinated infected individuals, viral RNA clearance seemingly proceeds at a faster rate in the former subjects.[3–6] Nevertheless, analyzes in these studies were not stratified by either time elapsed since full COVID-19 vaccination[3–5] or clinical status (asymptomatic vs. symptomatic) at the time of RT-PCR diagnosis.[3–6] Here, we add nuances to the aforementioned assumption by showing that among patients with breakthrough COVID-19, both the magnitude and dynamics of Delta variant RNA load in URT are markedly affected by time elapsed since full COVID-19 vaccination.

The current observational study included a convenience sample of 107 individuals (57 female; median age, 62 years; range, 50–94) who developed breakthrough COVID-19 at a median of 65 days (range, 17–199) after completion of the vaccination schedule with Comirnaty® (n = 73), Spikevax® (n = 13), Vaxzebria® (n = 12) or Janssen COVID-19 vaccine (n = 9). Diagnosis of SARS-CoV-2 infection was achieved by RT-PCR (TaqPath COVID-19 Combo Kit; Thermo Fisher Scientific, MS, USA)[2]) in nasopharyngeal specimens collected within the first 4 days after symptoms onset. SARS-CoV-2 RNA loads were estimated using the AMPLIRUN® TOTAL SARS-CoV-2 RNA Control (Vircell SA, Granada, Spain), and are reported as copies/ml throughout the study. Delta variant involvement was identified by whole-genome sequencing.

None of the participants in our sample had an immunosuppressive condition or was under immunosuppressive therapy at the time of symptoms onset. Most patients presented with mild disease (n = 105), whereas 16 had to be hospitalized (one in the intensive care unit). We arbitrarily stratified participants according to time elapsed between full vaccination and symptoms onset into three groups, which were balanced regarding participant numbers: Group 1: 15–45 days, Group 2: 46–90 days and Group 3: 91–200 days (Supplementary Table 1). Participants differed significantly across groups in age and hospitalization rate (older age and more hospital admissions in Group 3) and vaccine used, but were comparable regarding time interval between symptoms onset and RT-PCR diagnosis (median, 2 days) and sex. Taking the cohort as a whole, we noticed an increase in initial SARS-CoV-2 RNA loads in NP, positively associated with time since vaccination, irrespective of the vaccine used (Fig. 1A), although a clear trend towards lower initial SARS-CoV-2 RNA load was observed in participants in Group 1 compared to the other two groups. By analyzing SARS-CoV-2 RNA load trajectory over time since symptom onset (Fig. 1B) we observed a seemingly faster viral RNA load decay in patients in Group 1. To rule out bias related to vaccine used, we next performed a similar analysis restricted to subjects vaccinated with Comirnaty®, the vaccine used in the majority of participants in the current cohort (Supplementary Table 2). As depicted in Figure 2C, initial SARS-CoV-2 RNA loads gradually increased with time elapsed since vaccination, with significant differences in viral loads between participants in Groups 1 and 3 (P = 0.03). Again, viral load decrease appeared faster in patients in Group 1 than in the other two study groups (Fig. 1D).

Beyond the relatively modest sample size, our study has several further limitations. First, Delta variant peak RNA loads may have been reached before symptoms appeared, and not uniformly across participants in the study groups. Second, patients in Group 3 were much older than those in the remaining groups; it is possible that elderly individuals may exhibit different kinetics of SARS-CoV-2 Delta variant RNA load in URT, post-vaccination waning of immune responses, or both, compared to patients in the remaining study groups. Third, sequential NP specimens from participants were not available for analyzes.

Our data highlight the impact of time elapsed since full vaccination on the magnitude and decay kinetics of SARS-CoV-2 RNA loads in URT in COVID-19 patients during Delta variant breakthrough infections, and are in line with results published in a recent study[6]. How dissimilarities in viral loads translate into differences in infectiousness needs to be assessed. Disappearance of vaccine-elicited immune responses over time likely account for our

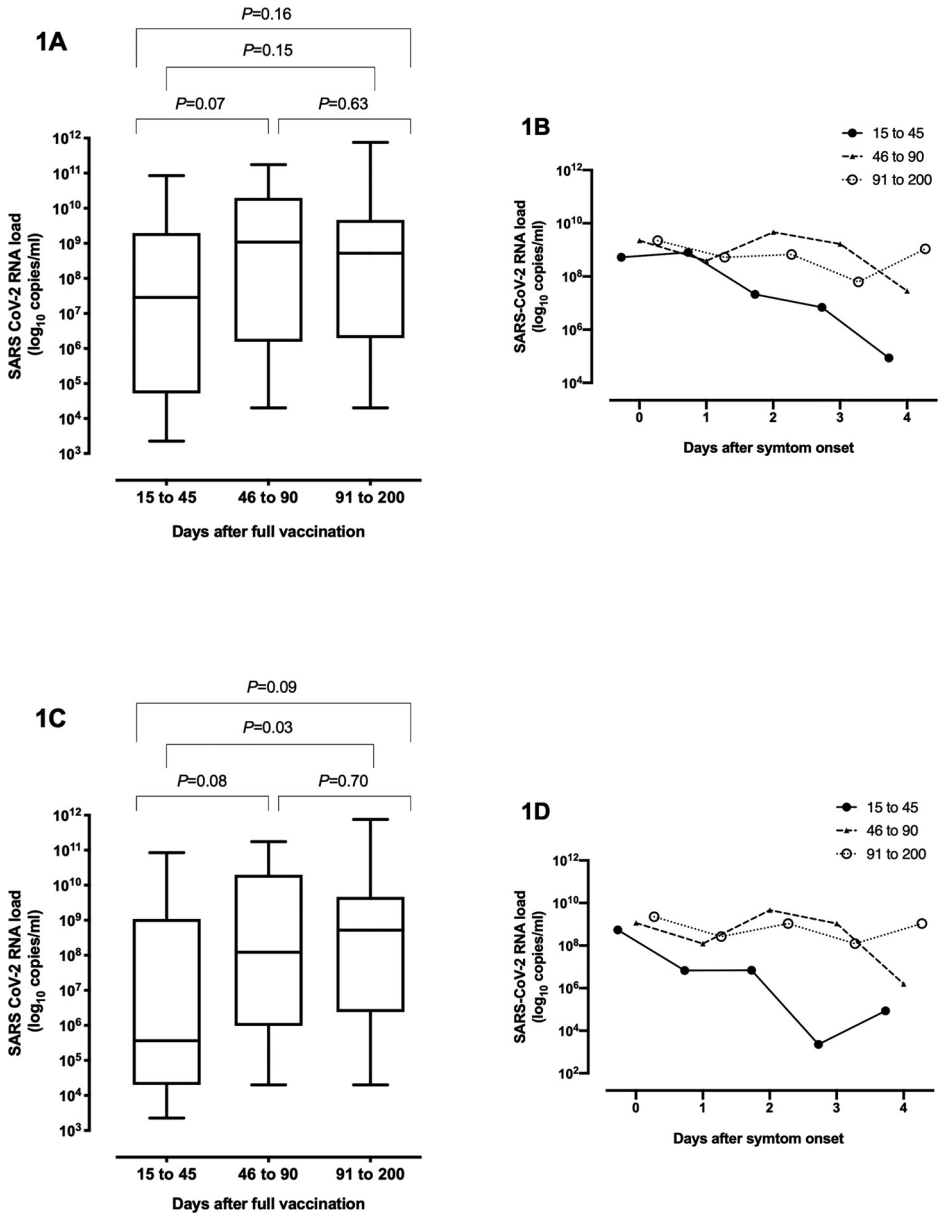

Fig. 1. SARS-CoV-2 Delta variant RNA load in the upper respiratory tract of individuals with breakthrough COVID-19 according to time elapsed since full vaccination. (A) Box-Whisker plot depicting initial SARS-CoV-2 RNA loads in all participants; (B) Kinetics of SARS-CoV-2 RNA load over the first 4 days after symptoms in all participants; (C) Box-Whisker plot depicting initial SARS-CoV-2 RNA loads in participants vaccinated with the Comirnaty® vaccine; (D) Kinetics of SARS-CoV-2 RNA load over the first 4 days after symptoms in participants vaccinated with the Comirnaty® vaccine. *P* values are shown for comparisons (Mann-Whitney U test or Kruskal-Wallis test, as appropriate).

findings, which should be verified in larger studies due to their potential public health implications.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.01.006.

References

1. Teyssou E., Delagrèverie H., Visseaux B., Lambert-Niclot S., Brichler S., Ferre V., et al. The delta SARS-CoV-2 variant has a higher viral load than the Beta and the historical variants in nasopharyngeal samples from newly diagnosed COVID-19 patients. *J Infect* 2021;**83**(4):e1–3 Oc1.
2. Costa R., Olea B., Bracho M.A., Albert E., de Michelena P., Martínez-Costa C., et al. RNA viral loads of SARS-CoV-2 Alpha and Delta variants in nasopharyngeal specimens at diagnosis stratified by age, clinical presentation and vaccination status. *J Infect* 2021;**S0163-4453**(21) 00641-1.
3. Chia P.Y., Xiang Ong S.W., Chiew C.J., Ang L.W., Chavatte J.M., Mak T.M., et al. Virological and serological kinetics of SARS-CoV-2 Delta variant vaccine-breakthrough infections: a multi-center cohort study. *Clin Microbiol Infect* 2021;**S1198-743X**(21) 00638-8.

4. Singanayagam A., Hakki S., Dunning J., Madon K.J., Crone M.A., Koycheva A., et al. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. *Lancet Infect Dis* 2021;**S1473-3099**(21)00648-4.
5. Siedner M.J., Boucau J., Gilbert R.F., Uddin R., Luu J., Haneuse S., et al. Duration of viral shedding and culture positivity with post-vaccination SARS-CoV-2 delta variant infections. *JCI Insight* 2021:e155483. doi:10.1172/jci.insight.155483.
6. Levine-Tiefenbrun M., Yelin I., Alapi H., Katz R., Herzel E., Kuint J., et al. Viral loads of Delta-variant SARS-CoV-2 breakthrough infections after vaccination and booster with BNT162b2. *Nat Med* 2021 Nov 2. doi:10.1038/s41591-021-01575-4.

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Regular and booster vaccination with inactivated vaccines enhance the neutralizing activity against Omicron variant both in the breakthrough infections and vaccinees

Dear Editor,

Recent articles in this Journal have reported the cross-neutralization of SARS-CoV-2 variants in individuals of vaccinees and convalescents, and compared the clinical features of Coronavirus disease 2019 (COVID-19) by SARS-CoV-2 Gamma variant based on a prospective cohort study of vaccinated and unvaccinated healthcare workers[1,2]. The emergence of SARS-CoV-2 variants with higher transmission ability and immune evasion against monoclonal antibodies, sera from natural infection and vaccine receipts, posing a serious threat to public health. On 26 November 2021, the World Health Organization named the new SARS-CoV-2 variant B.1.1.529 as Omicron, and defined it as a Variant of Concern (VOC). As the fifth VOCs currently, Omicron contains much more mutations in the Spike protein, including some mutations shown to reduce neutralizing efficiencies of COVID-19 vaccines in previous studies[3]. As the pandemic of COVID-19 has lasted for nearly two years, there are more and more convalescents either previously infected with wild-type SARS-CoV-2 or recently emerged variants. Meanwhile, booster vaccination of SARS-CoV-2 is now rapidly rolling out in China and other countries. Accordingly, neutralizing efficiency of convalescents and vaccinees with booster vaccination against the newly emerged Omicron variant needs further elucidation. Here we evaluated the neutralizing activity of plasma from the COVID-19 convalescents with wild type SARS-CoV-2 and different VOCs, and vaccinees with both regular vaccination and homologous booster of inactivated vaccine.

A total of 92 participants were included in this study, including 24 vaccinees with homologous booster vaccination (Vaccinee group), 26 individuals previously infected with wild type (WT) SARS-CoV-2 (WT group), 16 patients infected with Alpha variant (Alpha group, 12 patients received vaccination before infection), 6 patients infected with Beta variant (Beta group), and 20 patients infected with Delta variant (Delta group, 4 patients received vaccination before infection). All the individuals with breakthrough infection received at least one-dose inactivated vaccines (CoronaVac or BBIBP-CorV before laboratory confirmation of infection, and individuals in the vaccinee group received inactivated vaccines (BBIBP-CorV) and homologous booster vaccination. The detailed information was shown in **Table S1**. All the plasma samples in the WT group were collected over 386 days post illness onset/laboratory confirmation (median: 431.5; range: 386–471), and most samples (40/42, 95.23%) in the Alpha, Beta, Delta groups were collected over 7 days after laboratory confirmation (median: 18; range: 1–36). Samples from the vaccinee group were collected after the two dose of vaccination with median days of 85 (range: 28–103), and 95 days after booster vaccination.

For the WT group, about 46.15% (12/26) of the samples showed inhibition rates over 50% against Omicron variant at the first dilution (1:20). The geometric mean neutralizing titers (GMTs) against wild type SARS-CoV-2 (WT) and Omicron variant were 154.07 (54.64 to 1018.82), 22.96 (10 to 145.38), respectively, and the reduction fold was 6.71 for Omicron (**Fig. 1A**). This reduction is comparable with Mu variant while higher than Alpha, Beta, Gamma, Delta, Lambda variants[4]. For the Alpha group, all the samples from breakthrough infections showed detectable 50% inhibitory dose (ID_{50}) against Omicron variant, while 50% (2/4) were below the lower limit of quantitation in the naive infections. The overall GMTs against Alpha and Omicron variants were 7198.43 and 285 respectively, with 25.26-fold reduction for the Omicron variant (**Fig. 1B**). Comparison between breakthrough and naive infection showed significantly higher ID_{50} against both Alpha (9.33-fold) and Omicron variants (5.61-fold) in the breakthrough infection (**Fig. 1B**). Similar results were found in the Delta group, with all the samples from breakthrough infection showed detectable ID_{50} against Omicron variant, while 1 out of 16 was below the lower limit of quantitation in the naive infection. The overall GMTs against Delta and Omicron variants were 10,898.97 and 558.44, respectively, with 19.52-fold reduction for the Omicron variant (**Fig. 1D**). Significantly higher ID_{50} against both Delta (3.25-fold) and Omicron variants (1.77-fold) were also found in the breakthrough infections (**Fig. 1D**). For the Beta group, we only got the naive infections, and reduced neutralizing activities were also found with 18.38-fold reduction against the Omicron variant (**Fig. 1C**).

In the Vaccinee group, 91.66 (22/24) showed detectable ID_{50} against wild type SARS-CoV-2 at 95 days post booster vaccination with GMT of 299, and 1.67-fold, 14.52-fold, 3.72-fold and 6.73-fold reduction against Alpha, Beta, Gamma and Delta variants were found, respectively (**Fig. 2A**). Notably, only 25% (6/24) of the samples showed detectable ID_{50} against Omicron, with 16.07-fold reduction when compared with wild type SARS-CoV-2. We further compared the neutralization activity before and after booster vaccination against different VOCs. After booster vaccination, GMTs against wild type, Alpha, Beta, Gamma, Delta increased 13.47-fold,

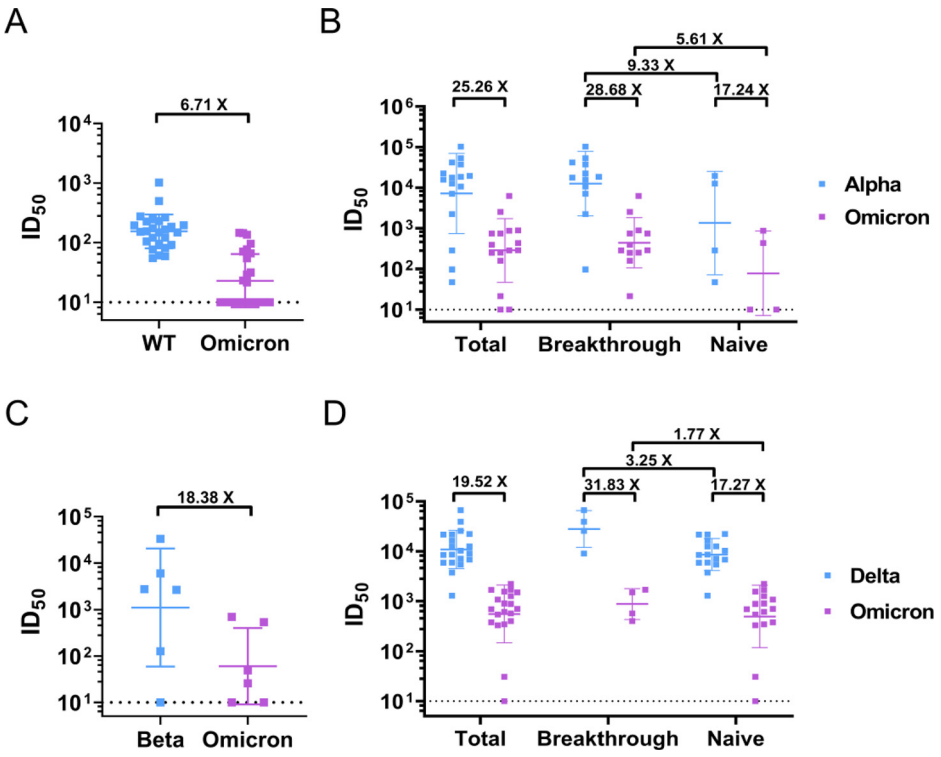

Fig. 1. Neutralizing activities of plasma samples from convalescents previously infected with wild type (A), Alpha (B), Beta (C) and Delta variants (D).

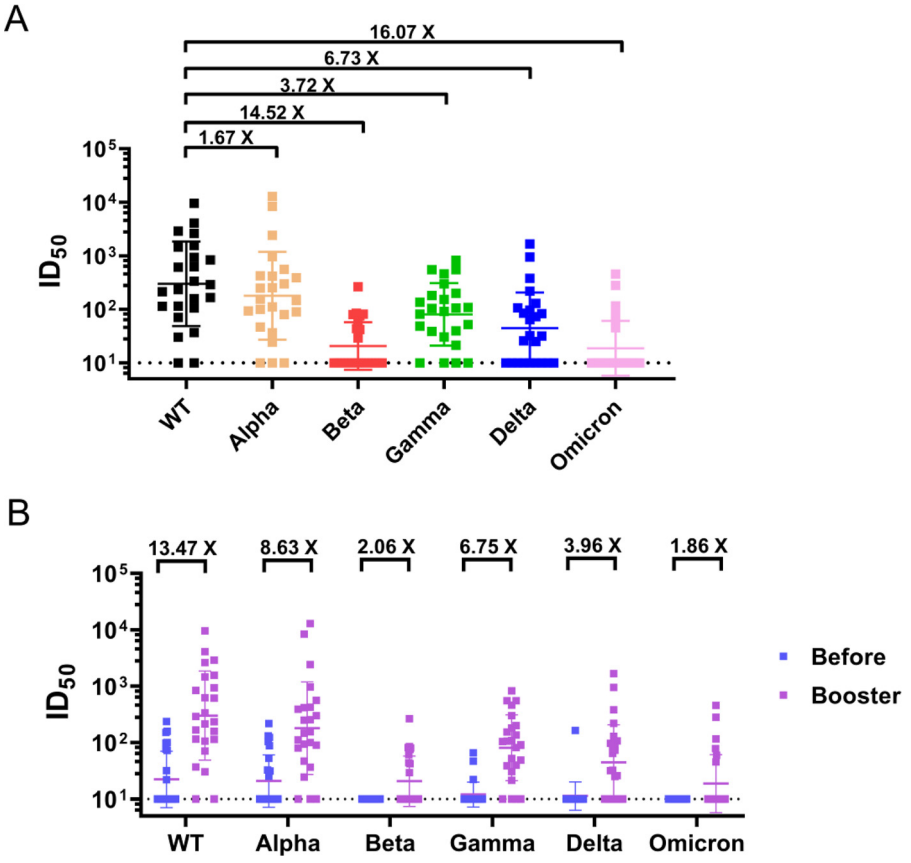

Fig. 2. Neutralizing activities of plasma samples from vaccinees with booster vaccination. A. ID_{50} of samples from vaccinees with booster vaccination against wild type, Alpha, Beta, Gamma, Delta and Omicron variants. B. Comparison of the neutralizing activities before and after booster vaccination.

8.63-fold, 2.06-fold, 6.75-fold and 3.96 fold, respectively (**Fig. 2B**). For the Omicron variant, all samples were below the lower limit of quantitation after the first two dose vaccination, similar with previous study[5]. However, 6 out of 24 (25%) showed detectable ID_{50} (GMT: 18.62; range: 10 to 454.4) after booster vaccination, with 1.86-fold increase in GMTs (**Fig. 2B**).

Although some recent studies have showed that Omicron variant could escape from the majority of existing SARS-CoV-2 neutralizing antibodies[6] and vaccine induced immune response[5], our study still provide unique information as follows. Firstly, we addressed whether COVID-19 convalescents previously infected with wild type SARS-CoV-2 could provide protection against Omicron Variant even after one year. Secondly, we used paired samples with both regular vaccination and homologous booster vaccination to evaluated the efficiency of booster vaccination. Thirdly, we compared the neutralizing activities against Omicron variant between breakthrough and naive infections with different VOCs.

Similar with previous studies, significant immune evasion was found for Omicron variant both in the convalescents and vaccinees, and the reduction folds was even higher than the previously least susceptible Beta and Mu variants[4,7], suggesting a higher risk of breakthrough infection or reinfection. It is worth noting that plasma samples collected over one year post infection showed lower reduction folds against Omicron variant when compared with other convalescents with different VOCs, which might correlate with the increase in the breadth and potency of SARS-CoV-2 specific antibodies due to the maturation of memory B cells[8-10]. Despite of the obvious immune evasion, our study also confirmed the importance of vaccination, both regular and booster vaccination. As shown in our study, breakthrough infections developed much higher neutralizing antibodies than the naive infection, both against the infected variant and Omicron variant. Moreover, booster vaccination could significantly increase the neutralizing responses to all the VOCs, although lower neutralizing abilities were found against some VOCs. Therefore, active vaccination still serve as one of the most effective measures to control the pandemic of COVID-19, and new vaccines with higher protective efficiency are also in urgent need.

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Declaration of competing interest

The authors have declared that no conflicts of interest exist.

Reference

1. Gidari A., et al. Cross-neutralization of SARS-CoV-2 B1.1.7 and P.1 variants in vaccinated, convalescent and P.1 infected. *J Infect* 2021;**83**:467–72. doi:10.1016/j.jinf.2021.07.019.
2. Luna-Muschi A., et al. Clinical features of COVID-19 by SARS-CoV-2 Gamma variant: a prospective cohort study of vaccinated and unvaccinated healthcare workers. *J Infect* 2021. doi:10.1016/j.jinf.2021.09.005.
3. Tao K., et al. The biological and clinical significance of emerging SARS-CoV-2 variants. *Nat Rev Genet* 2021;**22**:757–73. doi:10.1038/s41576-021-00408-x.
4. Yang; Y, et al. Longitudinal analysis of antibody dynamics in COVID-19 convalescents reveals neutralizing responses up to 16 months after infection. *Nature Mocribiol* 2021;**6**.
5. Lu L., et al. Neutralization of SARS-CoV-2 Omicron variant by sera from BNT162b2 or Coronavac vaccine recipients. *Clin Infect Dis* 2021. doi:10.1093/cid/ciab1041.
6. Cao Y, et al. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature* 2021;**585**.
7. Uriu K., et al. Neutralization of the SARS-CoV-2 Mu variant by convalescent and vaccine serum. *N Engl J Med* 2021;**385**:2397–9. doi:10.1056/NEJMc2114706.
8. Muecksch F., et al. Affinity maturation of SARS-CoV-2 neutralizing antibodies confers potency, breadth, and resilience to viral escape mutations. *Immunity* 2021;**54** 1853-1868.e1857. doi:10.1016/j.immuni.2021.07.008.
9. Sokal A., et al. Maturation and persistence of the anti-SARS-CoV-2 memory B cell response. *Cell* 2021;**184** 1201-1213.e1214. doi:10.1016/j.cell.2021.01.050.
10. Wang Z., et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* 2021;**595**:426–31. doi:10.1038/s41586-021-03696-9.

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Cross reactivity of spike glycoprotein induced antibody against Delta and Omicron variants before and after third SARS-CoV-2 vaccine dose in healthy and immunocompromised individuals

Dear Editor,

SARS-CoV-2 variants of concern threaten evasion of natural and vaccine-induced immunity. There is an urgent need to know how effective different vaccine strategies will be in reducing the transmission of and disease severity arising from the Omicron variant of concern (VOC). In the UK, two main vaccines have formed the basis of the national immunisation strategy: the AstraZeneca ChAdOx1 nCoV-19 vaccine (AZ)[1] and the Pfizer-BioNtech 162b2 COVID-19 vaccine (PFZ).[2] Both elicit immune responses directed against the original wildtype SARS-CoV-2 (Wuhan) spike glycoprotein and both have been shown to reduce the incidence of severe disease in clinical trials.[1,2] In mid-2021, the Delta VOC became dominant worldwide and led to the widespread deployment of booster immunisations using mRNA vaccines. In late November 2021, the Omicron VOC rapidly emerged displaying even greater transmissibility and has become the dominant SARS-CoV-2 virus in the UK and across

the world.[3] These rapid shifts in the pre-dominance of VOC outpaces and impairs the development and testing in clinical trials of new VOC-tailored vaccines. We do not yet know how well the different vaccine strategies applied in the UK will reduce the transmission of and severity of disease arising from rapidly emerging VOC in the general population and immunological vulnerable subgroups.

We have used the core design of an anti-IgG/A/M SARS-CoV-2 ELISA[4,5] to measure IgG antibodies specific for spike protein from the original Wuhan strain,[6] B.1.617.2 (Delta - Abingdon Health) and B.1.1.529 (Omicron - SinoBiological China). In addition, we assayed serial dilutions (250 – 7.8 IU/mL) of the WHO standard NIBSC 20/136[7] and the therapeutic monoclonal antibody therapy Sotrovimab (Glaxo Smith Kline) the base concentration of which is 62.5 mg/ml and a 500 mg dose is given to patients. We have used these ELISAs to determine cross-reactivity of spike glycoprotein induced antibody against Delta and Omicron variants before and after third SARS-CoV-2 vaccine dose.

We have recruited three well-characterised cohorts: firstly, a health care worker cohort from University Hospitals Birmingham from the *Determining the immune response to SARS-CoV-2 infection in convalescent health care workers (COCO)* study, who had PFZ as their primary two-dose vaccine course followed by PFZ booster (PPP cohort). Secondly, individuals classed as clinically extremely vulnerable (CEV) attending general practice for vaccination in Ulster, who had the AZ vaccine as their primary two-dose vaccine course followed by a PFZ third dose (AAP). Lastly, individuals on haemodialysis under renal care at the University Hospitals Birmingham; 70.3% of which had AZ as their primary course (HD cohort) followed by a PFZ third dose. Serum samples were taken 6 months following their second vaccination of their primary course, prior to the third dose with PFZ, and also 28 days following vaccination.

There was evidence of suboptimal seropositivity 6 months post primary dose of vaccination in all groups but particularly amongst HD and CEV patients. Samples taken 6 months post-primary vaccine course showed that for the HD cohort, 58.9% of individuals were seropositive against the Wuhan strain, 34.4% against Delta and 62.2% against Omicron strains. For the PPP cohort, seropositivity was maintained at 92.2% against Wuhan, 90% against Delta and 91.1% against Omicron strains. For the AAP cohort, seropositivity was 62.5% against Wuhan, 45.8% against Delta and 91.7% against Omicron strains (Fig. 1a-c).

Post third vaccine dose, there was a significant increase in the percentage of individuals who were seropositive and a rise in the median serum antibody concentration (as measured by optical density or OD) of these seropositive individuals. For the HD cohort, seropositivity was 98.8% against the Wuhan, 97.6% against Delta and 100% against Omicron strains. For the PPP and AAP cohorts seropositivity was 100% against all 3 strains . The increase in median OD following vaccination in HD patients was 2.46 to 2.98 ($p<0.0001$) for the Wuhan, 1.94 to 2.49 (non-significant (ns)) for Delta, and 1.37 to 2.84 ($p<0.0001$) for Omicron strains. The increase in median OD for the PPP cohort against the Wuhan strain was 2.62 to 2.88 (ns), for Delta 2.33 to 3.08 ($p<0.0001$) and for Omicron 1.71 to 3.37 ($p<0.0001$). For the AAP cohort, for the Wuhan strain the increase was from 2.38 to 3.52 ($p<0.0001$), for Delta 2.98 to 3.45 ($p = 0.0044$) and for Omicron 1.17 to 3.46 ($p<0.0001$). We also compared antibody concentrations following booster immunisation between the PPP and AAP groups: in the AAP group there was a higher median OD for the Wuhan (2.88 v 3.52, $p<0.0001$) and Delta strains (3.08 v 3.45, $p = 0.0022$) but not for the Omicron strain (3.37 v 3.46, ns) compared to the PPP group suggesting heterologous vaccine strategies may demonstrate enhanced immunogenicity against these SARS-CoV-2 variants.

Lastly, the WHO NIBSC 20/136 standard was run as a standard curve in the Wuhan, Delta and Omicron ELISAs and no significant loss of antibody binding was observed against any VOC (Supplementary Figure 1a). Similarly, a dilution series from 6.5 mg/ml to 0.003 mg/ml of Sotrovimab (GSK) found no loss of antibody binding against Omicron (Supplementary Figure 1b). Strong correlations exist between antibody binding and neutralisation[8] and between the presence of neutralising antibodies and protection against severe COVID-19.[9]

Understanding the pre-existing seroprevalence of antibodies directed against novel SARS-CoV-2 VOC and their induction following third-primary or booster immunisation is of critical importance in guiding public health policy during the ongoing SARS-CoV-2 pandemic. This knowledge is of particular relevance to immunologically vulnerable groups who either do not make a robust serological response to vaccination or fail to retain a serological response over time. In this study, we provide evidence supporting the need of a third dose of vaccination due to a waning antibody response at 6 months and the broadly cross-reactive humoral immunogenicity of the third vaccine dose against rapidly evolving SARS-CoV-2 VOC in healthy, CEV, and HD patients. However, it is important to note that antibody binding doesn't necessarily equate with functionality of antibodies, particularly in immunosuppressed individuals. Therefore, this is the best-case scenario and this study will need to be repeated with neutralisation assays going forward.

Ethical approval

The COCO study was ethically approved for this work by the London - Camden and Kings Cross Research Ethics Committee on behalf of the United Kingdom Health Research Authority – reference 20/HRA/1817. The Haemodialysis study was ethically approved for this work by the North West Preston Research Committee on behalf of the United Kingdom Health Research Authority for the National Institute of Health Research Coronavirus Immunological Analysis study – reference 20/NW/ 0240. The Ulster study was ethically approved for this work by the Office of Research Ethics Committee for Northern Ireland on behalf of the United Kingdom Health Research Authority - reference 20/WM/0184. The pre-2019 health controls was ethically approved for this work by the University of Birmingham Research Ethics Committee, United Kingdom - reference ERNE_16–178 (2002/201, Amendment Number 4).

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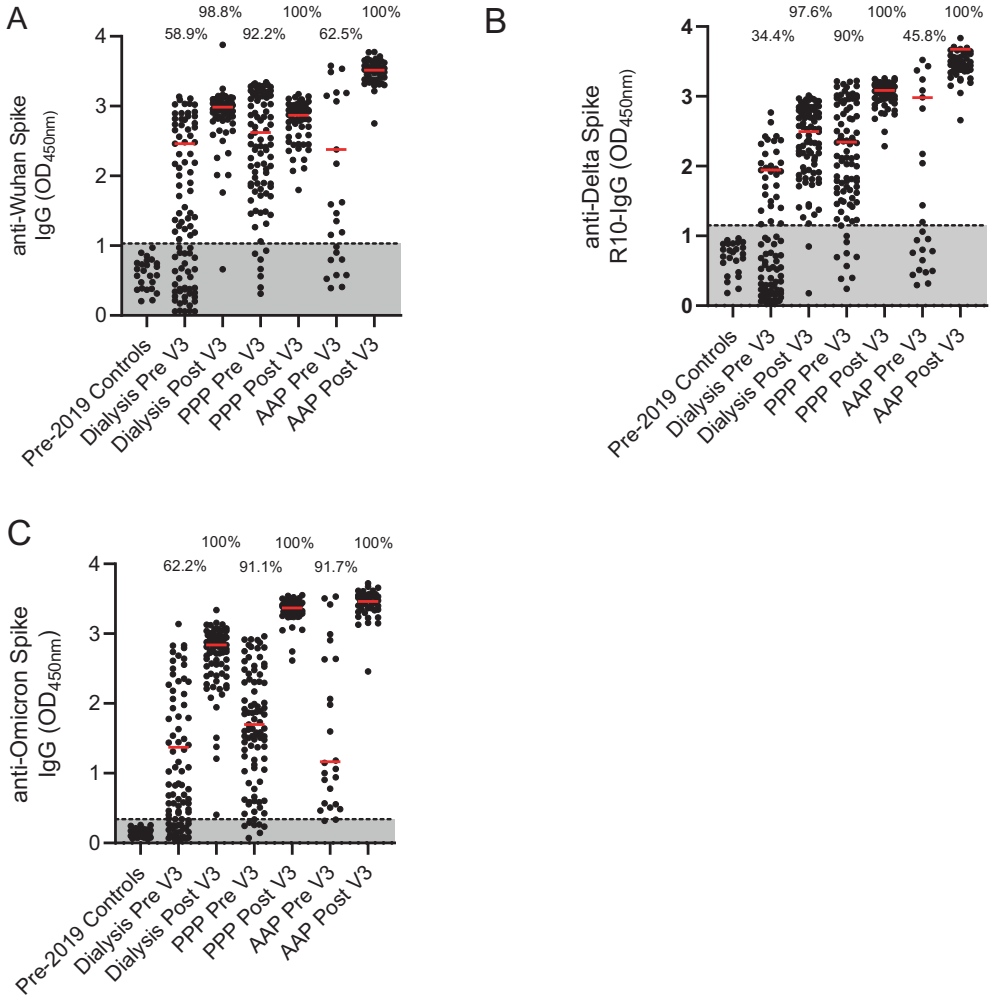

Fig. 1. Percentage of cohort with antibodies against Wuhan, Delta and Omicron strain.
A-Detection of anti- Wuhan spike IgG in pre-2019 controls, a dialysis population, a cohort of health care workers who have had three PFZ vaccines and a Clinically Extremely Vulnerable population in general practice who have had two AZ and one PFZ vaccine. Results are given for pre and post 3rd dose of vaccination. Percentage of cohort that are considered seropositive are included above the dot plots. The red line represents the median of the seropositive individuals in that cohort.
B-Detection of anti-Delta spike IgG for the same populations.
C-Detection of anti- Omicron spike IgG for the same populations
Pre- pre-3rd dose of vaccine and 6 months post 2nd dose. Post- 28 days post 3rd dose of vaccine. PPP- 3 Pfizer-BioNtech vaccines given in this cohort. AAP- two AstraZeneca ChAdOx1 nCoV-19 vaccines and the one Pfizer-BioNtech vaccine given in this cohort. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

COVID-19 Rapid Response Rolling Call. The COVID-HD Birmingham Study Group include Claire Backhouse, Anna Casey, Lynsey Dunbar, Beena Emmanuel, Megan Fahy, Alexandra Godlee, Paul Moss, Peter Nightingale, Liz Ratcliffe, Stephanie Stringer, Matthew Tabinor, Sian Faustini, Adam Cunningham, Alex Richter, Lorraine Harper. The PITCH study Group include Susanna Dunachie, Paul Klenerman, Lance Turtle, Thushan de Silva, Christopher Duncan, Rebecca Payne, Alex Richter, Ellie Barnes, Miles Carroll, Alexandra Deeks, Christina Dold.

Supplementary materials

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References

1. Voysey M., Clemens S.A.C., Madhi S.A., Weckx L.Y., Folegatti P.M., Aley P.K.. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS–CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021;**397**(10269):99–111 Jan.
2. Polack F.P., Thomas S.J., Kitchin N., Absalon J., Gurtman A., Lockhart S.. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020;**383**(27):2603–15 Dec.
3. England P.H. SARS-CoV-2 variants of concern and variants under investigation in England. Sage. 2021;(April).
4. Cook A.M., Faustini S.E., Williams L.J., Cunningham A.F., Drayson M.T., Shields A.M.. Validation of a combined ELISA to detect IgG, IgA and IgM antibody responses to SARS-CoV-2 in mild or moderate non-hospitalised patients. *J Immunol Methods* 2021:494 Jul 1.
5. Faustini S.E., Jossi S.E., Perez-Toledo M., Shields A.M., Allen J.D., Watanabe Y.. Development of a high-sensitivity ELISA detecting IgG, IgA and IgM antibodies to the SARS-CoV-2 spike glycoprotein in serum and saliva. *Immunology* 2021;**164**(1):135–47 Sep 1.
6. Watanabe Y., Allen J.D., Wrapp D., McLellan J.S., Crispin M.. Site-specific glycan analysis of the SARS-CoV-2 spike. *Science* 2020;**369**(6501) Jul 17330-3. Available from: https://pubmed.ncbi.nlm.nih.gov/32366695/.
7. NIBSC NI for BS andControls. WHO International Standard First WHO International Standard for anti-SARS-CoV-2 Immunoglobulin (human) NIBSC Code: 20/136 Instructions for Use (Version 2.0, Dated 17/12/2020). Potters Bar, Hertfordshire, EN6 3QG.
8. Earle K.A., Ambrosino D.M., Fiore-Gartland A., Goldblatt D., Gilbert P.B., Siber G.R.. Evidence for antibody as a protective correlate for COVID-19 vaccines. *Vaccine* 2021 Jul 22;**39**(32):4423–8 Available from: https://pubmed.ncbi.nlm.nih.gov/34210573/.
9. Khoury D.S., Cromer D., Reynaldi A., Schlub T.E., Wheatley A.K., Juno J.A..

Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;**27**(7):1205–11 Jul 1Available from: https://pubmed.ncbi.nlm.nih.gov/34002089/.

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Control of COVID-19 in China likely reduced the burden of multiple other infectious diseases

Dear Editor,

We read with great interest the analyses that suggested the control measures for COVID-19 likely reduced the infections of tuberculosis and influenza, but not the infections of human immunodeficiency virus and hepatitis C virus, possibly because these four diseases are transmitted through different routes.[1-3] In theory, the similar effects could be observed on multiple infectious diseases in China, because China also implemented strict and costly measures, such as lockdown of cities, mass restriction of travelling and gathering, mass isolation, mass mask wearing, and mass disinfection, for the control of COVID-19 caused by SARS-CoV-2 in 2020.[4] Here we provided data to test this theoretical inference that is important for calculating the benefits of the strict and costly measures for combating the COVID-19 pandemic.

Over 30 important infectious diseases were listed as Class A or B infectious diseases in China. The numbers of new cases of these diseases are reported monthly at the official website of National Health Commission of China (http://www.nhc.gov.cn/jkj/s3578/new_list.shtml). We collected these numbers of the years 2010−2020 from this official website. We excluded the numbers of the pandemic H1N1 subtype influenza, hepatitis D, human in-

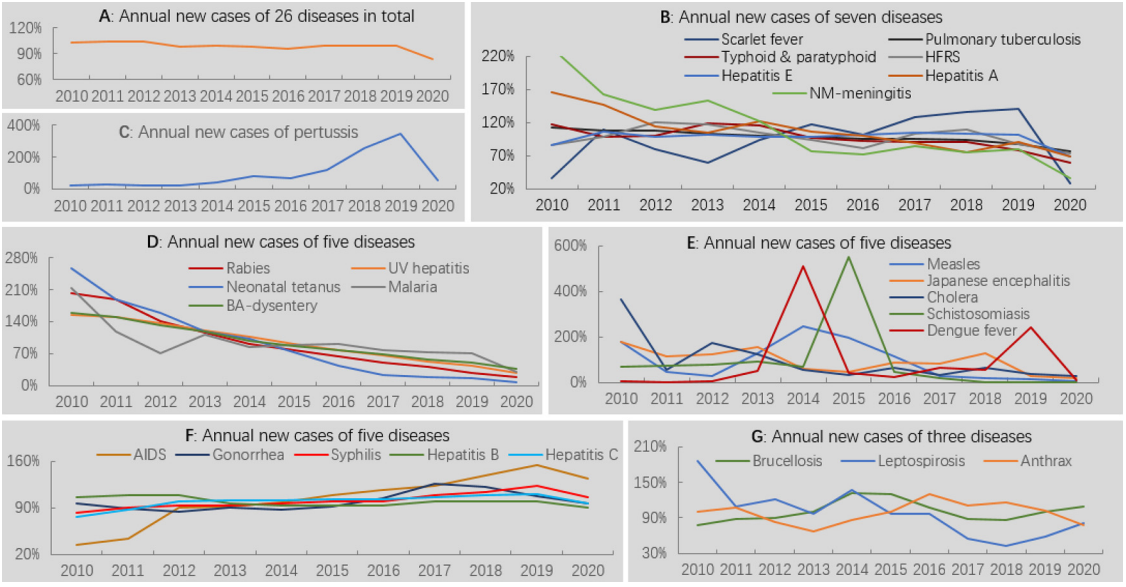

Fig. 1. Changes in the annual new cases of 26 infectious diseases in China over time compared with the relevant averages during the years 2010–2019. HFRS, hemorrhagic fever with renal syndrome; NM-meningitis, Neisseria meningitidis meningitis; UV-hepatitis, unclassified viral hepatitis; BA-dysentery, bacillary and amoebic dysentery; AIDS, acquired immune deficiency syndrome. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fection with H7 subtype avian influenza, and COVID-19, because these diseases were not listed as Class A or B infectious diseases in all the years 2010–2020. We also excluded the numbers of plague, severe acute respiratory syndrome (SARS), human infection with H5 subtype avian influenza, poliomyelitis, and diphtheria, because of low incidences of these diseases (<10 cases/year or <21 cases during the years 2010–2020).

We analyzed the numbers of the remaining 26 infectious diseases listed in Fig. 1, and statistical significance was calculated throughout using the T test compared with the averages of the years 2010–2019, if not specified.

The total new cases of these 26 major infectious diseases declined significantly by ~16.4% in China in 2020 ($P<0.05$) (Fig. 1A). The incidences of the five infectious diseases mainly transmitted through oral or nasal routes (IDMTTONRs), including scarlet fever, typhoid & paratyphoid, pulmonary tuberculosis, hemorrhagic fever with renal syndrome, and hepatitis E, declined significantly by 24.0 – 71.5% in 2020 ($P<0.05$ by the T test) (Fig. 1B). The incidences of two other IDMTTONRs, hepatitis A and Neisseria meningitidis meningitis, declined in 2020 significantly, as compared with the numbers of the years 2012–2019 ($P<0.05$ by the T test) (Fig. 1B). Pertussis, which is also an IDMTTONR, increased significantly in its incidence in China during the years 2010–2019 ($P<0.05$ by the Mann-Kendall test), sharing the same resurging trend with many other countries.[5] The incidence declined significantly in 2020 by 56.9 – 85.1% compared with the incidences in the years 2017–2019 (Fig. 1C).

The incidences of rabies, neonatal tetanus, malaria, bacillary and amoebic dysentery, and unclassified viral hepatitis declined by 28.7 – 57.7% in 2020 compared with the year 2019 (Fig. 1D). These changes possibly resulted from some reasons that existed before 2020, because they declined significantly during the years 2010–2019 ($P<0.05$ by the Mann-Kendall test).

The incidences of measles, Japanese encephalitis, cholera, schistosomiasis, and Dengue fever changed greatly during the years 2010–2019, and the incidences of these diseases all declined in 2020, although not significantly in statistics (Fig. 1E).

The incidences of the infectious diseases mainly transmitted sexually, through blood, or from domestic animals, including AIDS, gonorrhea, syphilis, hepatitis B, hepatitis C, brucellosis, leptospirosis, and anthrax, increased by from −9.9% to 34.3% without statistical significance ($P>0.05$) (Fig. 1F and Fig. 1G). The incidence of AIDS increased significantly during the years 2010–2019 ($P<0.05$ by the Mann-Kendall test), and this incidence kept relatively high in 2020 (Fig. 1F).

Together, changes of the incidences of the above 26 infectious diseases supported the theoretical inference that the strict and costly measures for the control of COVID-19 in China likely reduced significantly new cases of multiple IDMTTONRs, and they likely did not reduce new cases of infectious diseases mainly transmitted sexually, through blood, or from domestic animals. This is important for calculating the costs and benefits of the COVID-19 control measures and making rational decisions for pandemic control.

An analysis on the same topic was published using the data of two (2019–2020) or five (2016–2020) years, with the same conclusion on the IDMTTONRs and the contrary conclusion on infectious diseases mainly transmitted sexually, through blood, or from domestic animals.[6] By contrast, this report employed the data of 11 years (2010–2020), and therefore, this report revealed the longer trends of the analyzed diseases and excluded more confounding factors in making conclusions.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Lai C.C., Yu W.L.. The COVID-19 pandemic and tuberculosis in Taiwan. *J Infect* 2020;**81**(2):e159–61 PubMed PMID: 32534000. Pubmed Central PMCID: PMC7286835. Epub 2020/06/14. doi:10.1016/j.jinf.2020.06.014.
2. de Souza Luna L.K., Perosa D.A.H., Conte D.D., Carvalho J.M.A., Alves V.R.G., Cruz J.S., et al. Different patterns of Influenza A and B detected during early stages of COVID-19 in a university hospital in Sao Paulo. *J In-*

fect 2020;**81**(2):e104–5 PubMed PMID: 32445724. Pubmed Central PMCID: PMC7240256. Epub 2020/05/24. doi:10.1016/j.jinf.2020.05.036.
3. Chow A., Hein A.A., Kyaw W.M.. Unintended consequence: influenza plunges with public health response to COVID-19 in Singapore. *J Infect* 2020;**81**(2):e68–9 PubMed PMID: 32360879. Pubmed Central PMCID: PMC7192079. Epub 2020/05/04. doi:10.1016/j.jinf.2020.04.035.
4. Chen X., Gong W., Wu X., Zhao W.. Estimating economic losses caused by COVID-19 under multiple control measure scenarios with a coupled infectious disease-economic model: a case study in Wuhan, China. *Int J Environ Res Public Health* 2021;**18**(22):11753 PubMed PMID: 34831508. Pubmed Central PMCID: PMC8621982. Epub 2021/11/28. doi:10.3390/ijerph182211753.
5. Macina D., Evans K.E.. Bordetella pertussis in school-age children, adolescents and adults: a systematic review of epidemiology and mortality in Europe. *Infect Dis Ther* 2021;**10**(4):2071–118 PubMed PMID: 34435338. Pubmed Central PMCID: PMC8387212. Epub 2021/08/27. doi:10.1007/s40121-021-00520-9.
6. Bai B.K., Jiang Q.Y., Hou J.. The COVID-19 epidemic and other notifiable infectious diseases in China. *Microbes Infect* 2021;**24**(1):104881 PubMed PMID: 34419605. Pubmed Central PMCID: PMC8375246. Epub 2021/08/23. doi:10.1016/j.micinf.2021.104881.

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Performance of lateral flow assays for SARS-CoV-2 compared to RT-qPCR

Dear Editor,

We have read with interest the study by Lamb *et al.* on the real-world performance of a lateral flow immunoassay antigen device (LFD) for regular COVID-19 testing of hospital workers[1]. LFDs have found widespread application for broad-range screening, with subsequent confirmation of LFA-positive cases by RT-qPCR. While, in practice, an acceptably low number of positive LFD results turned out as false positives when validated with RT-qPCR, there is accumulating evidence that the number of positive cases missed, *i.e.*, false negative results obtained with LFDs is unacceptably high[2]. The LFD used in the study by Lamb et al. (Innova Medical Group, USA) had a high positive predictive value (PPV) when frequently used during periods of high prevalence of COVID-19. As the authors did not use the same sample for confirmation by RT-qPCR, however, it was not possible to directly link the test outcome to a certain cT value, or to evaluate the specificity[1].

We selected 10 common LFDs [*Abbott* (Panbio, Abbott Rapid Diagnostics Jena GmbH, Jena, Germany), *Acon* (Flowflex, Acon Laboratories Inc., CA, USA), Clungene (Hangzhou Clungene Biotech Co., Ltd., Tianjin, China), *Joysbio* (Joysbio (Tianjin) Biotechnology Co., Ltd., Tianjin, China), *Lepu* Medical (Beijing Lepu Medical Technology Co., Ltd., Peking, China), *Nadal* (nal von minden GmbH, Moers, Germany), *Orient* (Zhejiang Orient Gene Biotech Co., Ltd., Zhejiang, China), *Realy Tech* (Hangzhou Realy Tech Co., Ltd., Zhejiang, China), Roche (SD Biosensors, Gyeonggi-do, Republic of Korea), *Siemens* (Clinitest, Healgen Scientific Ltd., TX, USA)] and evaluated their performance using samples assigned for routine RT-qPCR screening of patients at the local university hospital between May and November 2021.

Fig. 1
Table 1

All clinical specimens were anonymized nasopharyngeal swabs in universal transport medium (119 positive and 55 negative) performed by trained personnel. All tests were performed in parallel using aliquots of the same samples. Samples were stored for a maximum of 48 h at 4 °C. The distribution of the cT-values of our samples was as follows: cT < 20, n = 16; 20–25, n = 31; 25–30, n = 31; 30–35; n = 29; >35; n = 12. Highest sensitivity was achieved by Nadal (79%), which however yielded the highest number of false positive results (31) and hence the lowest specificity (44%). Comparatively high sensitivity was achieved by Abbott (62%) and Orient (56%). Lowest sensitivity was achieved by Joysbio (33%) and Acon (33%). In the subset of samples with cT-values of < 25, sensitivity ranged from 68% to 91% (Nadal 94%). Siemens, Clungene and Acon showed no false positive results.

Binomial logistic regression analysis was applied to model the probability of obtaining a positive LFD result depending on the cT-value assuming 40 for PCR-negative tests or neglecting negative test results. We estimated the confidence intervals (CI) via non-parametric bootstrapping with 100.000 iterations.

In accordance with Lamb *et al.* and similar other studies, our study confirms that LFDs are suitable to identify potential super-spreaders with low cT values and to prevent large cluster formation, *e.g.*, in hospitals[1,3,4], however, regardless of the manufacturer, they are not likely to contribute significantly to control the infection dynamics caused by carriers of low viral load or by asymptomatic individuals, including those at early stages of infection.

It should be noted that cT values can vary among clinical laboratories, depending on the method for RNA extraction and the PCR kits used. As an example, in a study involving 123 participating certified laboratories in Austria including our lab, a variation in cT values of ± 4.7 cycles was observed[5].

Samples in our study were exclusively obtained via nasopharyngeal swabs by professionals, whereas other techniques, such as the common anterior nasal swab or incorrect swabbing by non-trained personal may significantly decrease *in vitro* virus concentration, and thus can increase the cT-values by several orders of magnitude[6-8] leading to a further loss of sensitivity. In the light of the repeated emergence of SARS-CoV-2 variants, one may also be worried about decreased sensitivity due to altered surface proteins, which are recognized be the LFD antibodies[9].

Based on our data, we conclude that LFDs cannot be recommended for general broad-range screening for SARS-CoV-2 infection in an asymptomatic population. At high infection dynamics however, insufficient PCR test capacities lead to logistic difficulties, an increased time-to-result, as well as decreased sensitivity related to sample pooling. In such settings, the short time-to-result of LFDs may support the timely identification and isolation of individuals with high viral load to dampen the dynamics of spread, as suggested by Lamb *et al.*[1].

Declaration of Competing Interest

The authors have no conflict of interest to declare.

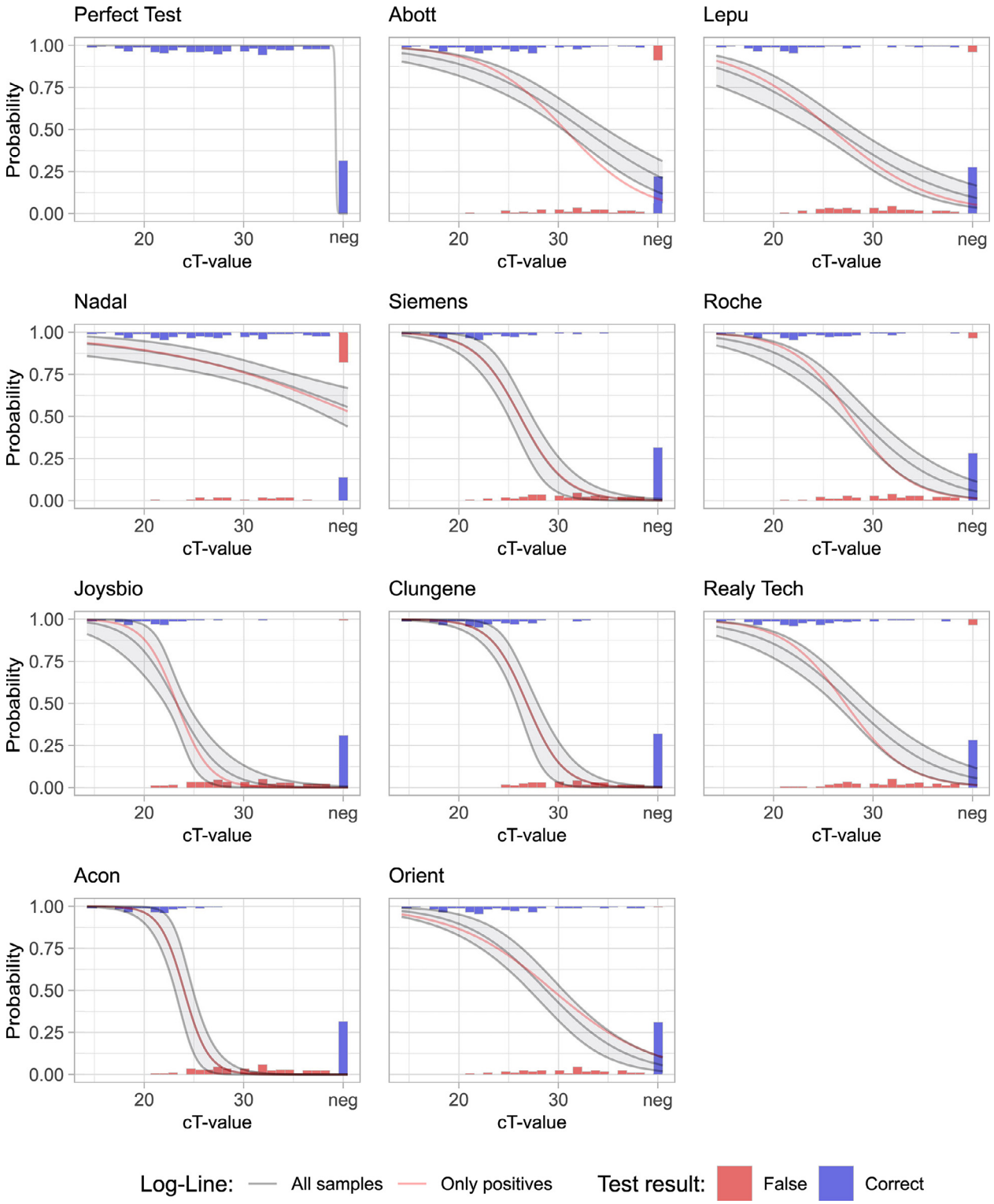

Fig. 1. illustrates the predicted LFD results based on cT values by RT-qPCR. The gray lines and areas indicate the predicted probability of a positive outcome and the 95% confidence derived from the logistic regression of all samples (imputed cT-value for PCR-negative samples = 40.0). The red line indicates the predicted probabilities of PCR-positive samples only. Bars on the top and on the bottom indicate positive and negative LFD test results, respectively. Red bars indicate false results, while correct results are indicated in blue.

Table 1
shows the cT thresholds at which 50% and 95% of the LFD test results become positive; in case of a50 and a95 all samples (imputed cT-value for PCR-negative samples = 40), in case of p50 and p95 only positive samples were considered. Brackets indicate the limit of the lower and upper 95% confidence interval.

LFA	TP	TN	FP	FN	a50	a95	p50	p95
Abott	76	38	15	43	32.1 [30.0; 34.3]	14.4 [14.0; NA]	30.2 [28.3; 32.5]	18.4 [14.7; NA]
Acon	39	55	0	80	23.5 [22.6; 24.4]	19.9 [18.4; 21.7]	23.5 [22.6; 24.4]	19.9 [18.4; 21.7]
Clungene	57	54	0	58	26.4 [25.4; 27.5]	21.0 [19.3; 23.1]	26.4 [25.4; 27.5]	20.9 [19.1; 23.0]
Joysbio	35	54	1	84	22.8 [21.6; 23.8]	16.2 [14.1; NA]	22.8 [21.8; 23.8]	18.0 [15.4; 20.4]
Lepu	53	48	7	66	25.7 [23.2; 27.8]	NA	25.4 [23.4; 27.6]	NA
Nadal	94	24	31	25	40.0 [NA; NA]	NA	40.0 [34.5; NA]	NA
Orient	69	54	1	50	28.3 [26.6; 30.0]	16.3 [14.1; NA]	29.0 [26.9; 31.7]	14.0 [13.9; NA]
Realy.Tech	59	49	6	60	27.4 [25.7; 29.0]	14.3 [13.9; NA]	26.7 [25.4; 28.3]	17.6 [14.5; NA]
Roche	62	49	6	57	27.9 [26.3; 29.6]	15.6 [14.0; NA]	27.2 [25.8; 28.7]	18.6 [15.4; 21.7]
Siemens	53	55	0	66	25.7 [24.5; 26.9]	19.0 [16.6; 21.6]	25.7 [24.5; 26.9]	18.9 [16.2; 21.5]

Author contributions

MP, SH and JH conceived the study and designed methodology; ME and MW provided clinical supervision of the study. CB, JZ, TE and SH collected the data; MP, RE and JH analysed the data; MP, SH and VW led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Ethical statement

The study was approved by the Ethics Committee of the Danube University Krems on May 28th, 2021.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.01.013.

References

1. Lamb G.. Real-world evaluation of COVID-19 lateral flow device (LFD) mass-testing in healthcare workers at a London hospital; a prospective cohort analysis. *J Infect* 2021;**83**:452–7.
2. Armstrong S.. Covid-19: tests on students are highly inaccurate, early findings show. *BMJ* 2020;**371**:m4941.
3. Avadhanula V.. Viral load of severe acute respiratory syndrome coronavirus 2 in adults during the first and second wave of coronavirus disease 2019 pandemic in Houston, Texas: the potential of the superspreader. *J Infect Dis* 2021;**223**:1528–1537.
4. Krone, M., Noffz, A., Richter, E., Vogel, U. & Schwab, M. Control of a COVID-19 outbreak in a nursing home by general screening and cohort isolation in Germany, March to May 2020. *Eurosurveillance*26, 2001365 (2021).
5. Buchta C.. A look at the precision, sensitivity and specificity of SARS-CoV-2 RT-PCR assays through a dedicated external quality assessment round. *Clin Chem Lab Med* 2021. doi:10.1515/cclm-2021-1004.
6. Hanson K.E.. Self-collected anterior nasal and saliva specimens *versus* health care worker-collected nasopharyngeal swabs for the molecular Detection of SARS-CoV-2. *J Clin Microbiol* 2020;**58** e01824-20.
7. Richard-Greenblatt M.. Quantifying the impact of nasopharyngeal specimen quality on severe acute respiratory syndrome coronavirus 2 test performance. *Open Forum Infect Dis* 2021;**8** ofab235.
8. Zhou Y., O'Leary T.J. Relative sensitivity of anterior nares and nasopharyngeal swabs for initial detection of SARS-CoV-2 in ambulatory patients: rapid review and meta-analysis. *PLoS ONE* 2021;**16**:e0254559.
9. Karim S.S.A., Karim Q.A.. Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. *Lancet* 2021;**398**:2126–8.

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SARS-CoV-2 RNA loads in Vietnamese children

Dear Editor,

A recent report from Spain in your journal reported that SARS-CoV-2 viral loads in children were lower than that in adults[1], consistent with findings from previous studies.[2,3] However, others have documented comparable SARS-CoV-2 RNA loads between children and adults.[4,5] In addition to these inconsistent findings, the role of children as drivers of SARS-CoV-2 transmission remains poorly understood.[6] Therefore, better understanding of SARS-CoV-2 RNA loads in children, and the prevalence of children among those with SARS-CoV-2 infection remains critical to inform infection control measures. Here, we focused our analysis on SARS-CoV-2 prevalence and RNA loads in children and adults detected in Da Nang city, central Vietnam between July 1, 2021 and September 30, 2021.The present study formed part of the national COVID-19 outbreak response. Accordingly, obtaining inform consent from the study participants were deemed unnecessary.

Central to the COVID-19 outbreak response in Da Nang city has been a cost-effective sample pooling strategy for mass screening of SARS-CoV-2.[7,8] Accordingly, SARS-CoV-2 testing was performed on any contacts of a confirmed case, regardless of clinical status, and on all those living in areas of the city with ongoing community transmission or arriving in Da Nang city from other provinces/cities with on-going community transmission.

We extracted available demographic information and data on Ct values of SARS-CoV-2 RT-PCR obtained from the aforementioned community screening. To identify the responsible SARS-CoV-2 variant, a convenient sample of nasopharyngeal swabs with sufficient volume and viral loads were whole-genome sequenced using ARTIC3 protocol as previously described.[9]

During the study period, Da Nang city experienced an outbreak of COVID-19 with 4590 PCR-confirmed cases detected after testing over 3.3 million people. The majority of cases were detected in August and early September 2021 (**Fig. 1**). Information about demographic and SARS-CoV-2 RT-PCR Ct values was available from 3040 individuals, accounting for 66.2% of the reported cases. The 3040 study participants included 1408 (46.3%) males and 1632 (53.7%) females, and were aged from 1 to 97 years (median: 36 years). 629 were children (median in years: 11, range, 1–17 years), accounting for 20.7% of the total PCR-confirmed cases included for analysis.

The SARS-CoV-2 RT-PCR Ct values of the whole group ranged between 10 and 39 (median: 20). Compared with adults, children had higher Ct values (i.e. lower RNA loads): median (range): 20 (13–39) vs. 19 (10–39) ($p<0.001$) (**Fig. 2A**). Among adults but not children, females had higher RNA loads than males: median Ct value (range): 19 (11–39) vs. 20 (10–39) ($p<0.001$) (**Fig. 2B**). Among children, lower Ct values (i.e. higher RNA loads) were detected among those between 0 to 1 and 12–17 years old than those between 5 and 11 years old: median (range): 20 (13–37), 21 (14–39) and 19.5 (14–37) respectively ($p<0.003$) (**Fig. 2C**).

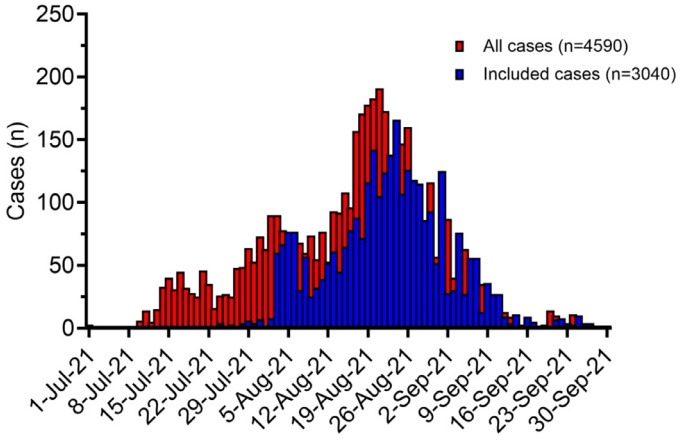

Fig. 1. Epidemic curve depicting the number of cases tested positive for SARS-CoV-2 between July 1, 2021 and September 30, 2021 in Da Nang City (Red) and the number of cases included for analysis during the same period (blue) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

A total of 64 SARS-CoV-2 whole-genome sequences were obtained, including 16 (25%) from children and 48 (75%) from adults. All were assigned to the SARS-CoV-2 Delta variant (sub-lineage AY57) by Pangolin, and lineage 21I by Nextstrain (**Supplementary Fig. 1**). The lineage 21I, including sub-lineage AY57, is one of the three reported clades of the Delta variant. Details about the genetic diversity of SARS-CoV-2 Delta variant detected across Vietnam will be described in a separate report.

Here, we showed that children accounted for a substantial proportion of PCR confirmed cases of SARS-CoV-2 infection detected through mass screening in Da Nang city, Vietnam. Consistent with previous reports,[2,3] we showed that children carried lower RNA loads than adults did. Yet, 50% of the infected children across age groups had a Ct value of ≤21 (i.e. high RNA loads). Although we did not perform virus culture to demonstrate the presence of live virus in PCR positive samples, previous studies showed that Ct values <24 were highly predictive of virus culture positivity (i.e. infectiousness).[2,10] These data suggested that children might play an important role in the transmission of SAR-CoV-2. Because the current COVID-19 vaccination program does not cover children below 5 years old, the rapid vaccine deployment worldwide may make the role of young children in the transmission of SARS-CoV-2 more important in the near future. The observed higher RNA loads in female adults than in male adults merits further research, in particular whether high viral burden is associated with more severe disease.

Our sequencing results have expanded the geographic distribution of the SARS-CoV-2 variant 21I, one of the three reported clades of the Delta variant. SARS-CoV-2 Delta variant accounted for 100% of the SARS-CoV-2 whole-genome sequences from Vietnam deposited to GISIAD since June 2021 (gisaid.org). Therefore, the demographic and virological features described here might be extrapolated for SARS-CoV-2 Delta variant infection in Vietnamese people.

The strength of our study includes that we included the majority of cases tested positive through mass screening for analysis regardless of the clinical presentations. As such, our data more accurately reflect the RNA loads of SARS-CoV-2 in children, whether or not they develop symptoms. Indeed, the proportion of children among SARS-CoV-2 PCR confirmed cases in the present study was comparable with that from a recent community-based study in the USA.[4] However, since we did not follow up the participants during quarantine/hospitalization, we were not able to assess the kinetics of RNA loads over the course of the infection. A previous study showed children had a faster SARS-CoV-2 clearance than adults.[5] Additionally, we were not able to compare the RNA loads between asymptomatic and symptomatic infections in children, although a previous report demonstrated that these two groups had comparable RNA loads.[5]

In summary, we report that children accounted for 20.7% of RT-PCR confirmed cases of SARS-CoV-2 infection detected during a community outbreak in Da Nang City, Vietnam. High RNA loads (i.e. Ct values of ≤21) were recorded in half of the infected children across age groups. The contribution of children, especially those not eligible for the current COVID-19 vaccination program, to the transmission dynamics of SARS-CoV-2 requires further research.

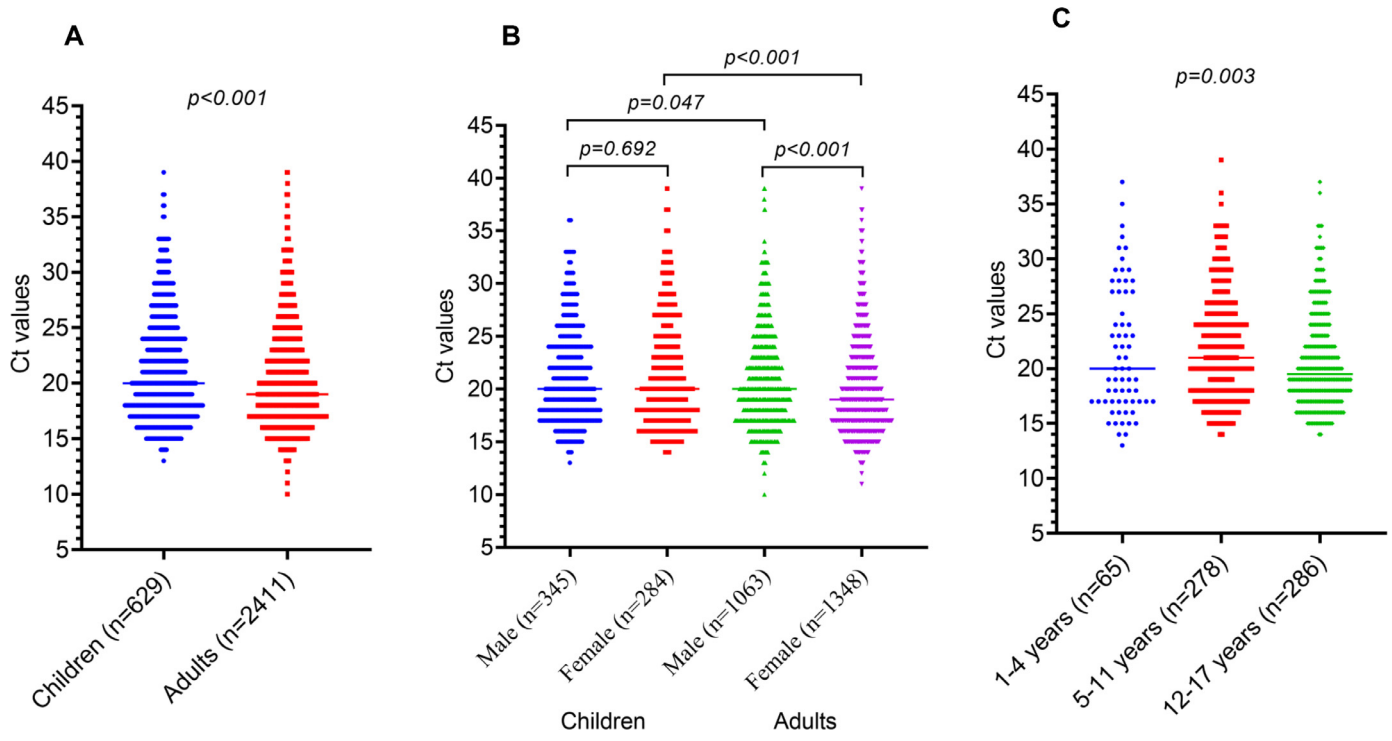

Fig. 2. SARS-CoV-2 RNA loads comparison between children and adults (A), between males and females (B), and subgroups of children (C) Mann-Whitney U test was used two compared between two groups (panel A and B), and Kruskal-Wallis test was used to compare between three age groups among children (panel C).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.01.010.

References

1. Cendejas-Bueno E., et al. Lower nasopharyngeal viral loads in pediatric population. The missing piece to understand SARS-CoV-2 infection in children? *J Infect* 2021;**83**(2):e18–19.
2. Bullard J., et al. Infectivity of severe acute respiratory syndrome coronavirus 2 in children compared with adults. *CMAJ* 2021;**193**(17):E601–6.
3. Jones T.C., et al. Estimating infectiousness throughout SARS-CoV-2 infection course. *Science* 2021;**373**(6551):1–13.
4. Chung E., et al. Comparison of symptoms and RNA levels in children and adults with SARS-CoV-2 infection in the community setting. *JAMA Pediatr* 2021;**175**(10):e212025.
5. Costa R., et al. Upper respiratory tract SARS-CoV-2 RNA loads in symptomatic and asymptomatic children and adults. *Clin Microbiol Infect* 2021;**27**(12) 1858 e1-1858 e7.
6. WHO. *Science Brief: COVID-19 Disease in Children and Adolescents*. WHO; 2021.
7. Thanh T.T., et al. The application of sample pooling for mass screening of SARS-CoV-2 in an outbreak of COVID-19 in Vietnam. *Am J Trop Med Hyg* 2021:1531–4.
8. Van Tan L., COVID-19 control in Vietnam. *Nat Immunol* 2021;**22**(3):261.
9. Chau N.V.V., et al. Rapid whole-genome sequencing to inform COVID-19 outbreak response in Vietnam. *J Infect* 2021;**82**(6):276–316.
10. Bullard J., et al. Predicting infectious severe acute respiratory syndrome Coronavirus 2 from diagnostic samples. *Clin Infect Dis* 2020;**71**(10):2663–6.

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