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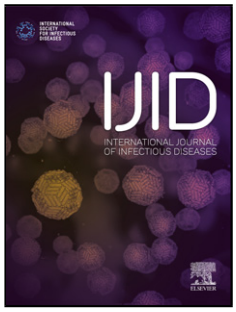

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High prevalence of pre-existing serological cross-reactivity against SARS-CoV-2 in sub-Sahara Africa

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Highlights

● High prevalence of serological cross-reactivity against SARS-CoV-2 in pre-COVID-19 pandemic plasma samples from sub-Sahara Africa.

● Pre-COVID-19 pandemic plasma displayed strong reactivity against other human coronaviruses.

● Exposure to other coronaviruses may induce cross-reactive antibodies against SARS-CoV-2 in sub-Sahara Africa.

Abstract

Objective: Significant morbidity and mortality from SARS-CoV-2 has been experienced in America, Europe and Asia; whereas, the number of infections and deaths in sub-Sahara Africa (SSA) has remained comparatively low. One hypothesis is that population in SSA has been exposed to other coronaviruses prior to the COVID-19 pandemic and resulted in some degree of cross-protection against SARS-CoV-2 infection and pathogenesis. Our goal was to evaluate this hypothesis by comparing SARS-CoV-2 cross-reactive antibodies in pre-pandemic plasma samples collected from SSA and USA.

Method: Pre-COVID-19 pandemic plasma samples from SSA and USA were collected and tested by immunofluorescence assay against the spike and nucleocapid proteins of all known human coronaviruses (HCoV).

Results: Significantly higher prevalence of SARS-CoV-2 serological cross-reactivity was detected in samples from SSA compared to USA. Majority of these cross-reactive samples cross-recognized SARS-CoV-2 nucleocapsid protein together with recognition of spike proteins from other HCoVs. Since nucleocapsid proteins from HCoV-NL63 and HCoV-229E were detected by majority of samples, it implicates prior exposure to these two HCoVs as the likely source for cross-reactive antibodies against SARS-CoV-2.

Conclusion: Low SARS-CoV-2 infection and disease in SSA appears to correlate with pre-pandemic serological cross-recognition of HCoVs, which are substantially more prevalent in SSA than USA.

Keywords: SARS-CoV-2; COVID-19; cross-reactivity; sub-Sahara Africa; serology; human coronavirus; HCoV-NL63; HCoV-229E.

Introduction

Since the first case of the COVID-19 pandemic was reported in Wuhan, China in late 2019, its causative agent severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has spread rapidly worldwide (Lu et al., 2020). SARS-CoV-2 is a betacoronavirus and a close relative to the original SARS and Middle East respiratory syndrome coronavirus (MERS) which both cause lethal diseases in human (Chen et al., 2020, Gussow et al., 2020). There are four other less pathogenic human coronaviruses (HCoV), HCoV-OC43, HCoV-HKU-1, HCoV-NL63 and HCoV-229E that cause mild upper respiratory tract disease referred to as the “common cold” (Chen et al., 2020, Gussow et al., 2020).

At the time of writing, the COVID-19 pandemic has resulted in over 31 million confirmed SARS-CoV-2 infections and nearly a million deaths, with the USA alone contributing nearly 22% of the confirmed cases and 21% of the confirmed deaths (Nuzzo et al., 2020). A number of factors would lend support to the expectation that populations in sub-Sahara Africa (SSA) might be more susceptible to coronaviral infection and disease. These include the high infectious disease burden (Ebola, yellow fever and cholera outbreaks as well as endemic high prevalence HIV-1, tuberculosis, malaria and parasitic diseases), a multiplicity of socioeconomic factors, poor hygiene and nutritional sufficiency, and lack of health care access in rural areas (Oleribe et al., 2015, Semeere et al., 2016). While infrastructure for diagnostics and epidemiological surveillance is suboptimal in Africa, where large scale testing has been possible the COVID-19 case mortality rates are lower than elsewhere in the world. There are no reports of any abnormal increase in the number of respiratory diseases or deaths – the hallmark of the COVID-19 pandemic - in SSA. Despite the high number of COVID-19 cases and case-mortality in America, Europe and Asia, the COVID-19 disease burden in SSA has remained surprisingly low (Nuzzo et al., 2020). A potential factor could be the relatively younger African populations as compared to that in America or Europe. This may result in more asymptomatic cases (Gaye et al., 2020). Additionally, if the onerous high infectious disease burden in SSA includes exposure to human coronaviruses, this could elicit humoral responses against conserved epitopes among coronaviruses that might engender cross-protection. This prior exposure to other coronaviruses may offer some level of cross-protective immune responses against SARS-CoV-2 infection, thereby, reducing either the number and/or severity of COVID-19 cases.

To investigate this hypothesis, we examined pre-COVID-19 pandemic Tanzanian, Zambian and USA plasma samples for serological cross-reactivity against the spike and

nucleocapsid proteins of SARS-CoV-2 and other human coronaviruses (SARS, MERS, HCoV-OC43, HCoV-HKU-1, HCoV-NL63 and HCoV-229E), as well as whether HIV-1 infection, which is endemic in SSA, could affect the prevalence of serological cross-reactive against SARS-CoV-2. We found that pre-COVID-19 pandemic sub-Saharan African samples have a significantly higher prevalence of serological cross-reactivity against SARS-CoV-2 than samples from the USA. Additionally, SARS-CoV-2 cross-reactive plasma samples strongly recognized the spike and nucleocapsid proteins from specific human seasonal coronaviruses, suggesting prior exposure to these other coronaviruses may induce partially protective responses against SAR-CoV-2.

Material and methods

Study cohort and samples

The study cohort was comprised of 289 consenting subjects, ≥18 years of age and of both genders from Dar es Salaam, Tanzania; Lusaka, Zambia; and Lincoln, Nebraska, USA. The Tanzanian samples included 105 plasma samples collected from voluntary blood donors collected between March and May of 2019. Zambian samples included 99 plasma samples collected between 2017 and early 2019. USA plasma samples were from 85 blood donors collected in 2005, 2007 and 2009 in Lincoln, Nebraska and were also evaluated for comparison. All study procedures were approved by the institutional review boards from the Tanzania National Institute for Medical Research, Ocean Road Cancer Institute, University of Zambia Biomedical Research Ethics Committee, and the University of Nebraska-Lincoln.

HIV serological testing

HIV-1 serology was determined by HIV Rapid Test Algorithm (United Republic of Tanzania, 2007) in Tanzania and Alere Determine HIV-1/2 Ag/Ab Combo test in Zambia. The serological results were verified in our lab at Lincoln, Nebraska using HIV-1-2.0 First Response kit (Premier Medical Corporation Limited, Daman, India).

Immunofluorescence assay against SARS-CoV-2 and other human coronaviruses

To detect the presence of serological cross-reactivity against SARS-CoV-2 and other human coronaviruses, we used an immunofluorescence assay (IFA) against the spike and nucleocapsid proteins of SARS, SARS-CoV-2, MERS, HCoV-OC43, HCoV-HKU-1, HCoV-NL63 and HCoV-229E. Briefly, HEK-293T cells (ATCC, USA) were transfected with mammalian expression plasmids encoding either the spike or nucleocapsid proteins of the respective coronaviruses (Addgene and Sino Biological, USA). After 48-hours, the transfected cells were fixed and seeded onto 12-well polytetrafluoroethylene (PTFE) printed slides (Electron Microscopy Sciences, USA) where each well contained either spike, nucleocapsid or mock transfected cells and followed by permeabilization with 0.3% H_2O_2 methanol solution. The prepared IFA slides were stored at -80℃.

Plasma samples were diluted 1:20 with PBS, 0.1% Tween-20 and incubated at room temperature for 30 minutes. The prepared IFA slides were thawed and incubated with PBS, 0.1% Tween-20 for 30 minutes at 37℃. Each diluted plasma sample was then added onto cells expressing each HCoV antigen or control wells and incubated for 1 hour at 37℃. Following primary antibody binding and washing, secondary mouse monoclonal anti-human IgG antibody (ATCC, USA) was bound for 1 hour at 37℃ followed by washes to remove excess unbound antibodies. Tertiary CY2-conjugated donkey anti-mouse IgG (Jackson Immuno Research Laboratories, USA) was then added and incubated for 1 hour at 37℃. Finally, the slides were

counterstained with 0.004% Evans blue solution for 30 seconds. All IFA slides were washed three times with PBS after each incubation step. The stained IFA slides were read by three independent readers on a Nikon Eclipse 50i fluorescence microscope. Positive cells were enumerated by green fluorescence against a red cellular counterstain. A well was only considered positive or negative if at least two independent readers were concordant in reporting the outcome. Summarized results and statistical analysis (two-tailed Fishers' exact test) were conducted and plotted using GraphPad (GraphPad Software, USA).

Results

To evaluate the serological cross-reactivity against SARS-CoV-2 and other human coronaviruses, we obtained blood donor plasma samples from Tanzania (n = 105), Zambia (n = 99) and the USA (n = 85) (Table 1). These samples were collected between 2005 to May 2019 and therefore are from prior to the current COVID-19 pandemic; however, due to the retrospective nature of the study, synchronously sampled plasma were not available. Among our cohort, 6.7% and 43.4% of the Tanzania and Zambia samples, respectively, were HIV-1 positive. Whereas all plasma samples collected in the USA were HIV-1 negative. The high prevalence of HIV-1 infection in the Zambian samples does not reflect the national HIV-1 infection rates, but rather was intended to support comparison of cross-reactivity against SARS-CoV-2 and recognition of other coronaviruses between HIV-1 positive and negative subjects.

The plasma samples were screened for cross-reactivity against SARS-CoV-2 using IFA. As shown in figure 1, COVID-19 convalescent positive control plasma resulted in strong green fluorescence staining in cells expressing either SARS-CoV-2 spike or nucleocapsid proteins, but

not in mock transfected cells. There was no green fluorescence evident on antigen-expressing cells stained with negative control plasma, demonstrating the specificity of IFA to detect SARS-CoV-2 specific IgG antibodies. Interestingly, green fluorescence was evident on cells expressing either SARS-CoV-2 spike or nucleocapsid proteins when stained with some pre-COVID-19 pandemic plasma samples. This result indicates the presence of antibodies cross-reactive against SARS-CoV-2 prior to the current COVID-19 pandemic (Figure 1). Compared to samples from the USA (2.4%), the prevalence of serological cross-reactivity against SARS-CoV-2 was significantly higher in Tanzania (19%) ($P = 0.0002$) and Zambia (14.1%) ($P = 0.0069$) (Figure 2A). A breakdown of the anti-SARS-CoV-2 cross-reactivity indicates that most of the Tanzanian and Zambian cross-reactive responses targeted the SARS-CoV-2 nucleocapsid protein, 17.1% ($P = 0.0001$) and 13.1% ($P = 0.0018$), respectively, levels significantly higher than in samples from the USA 1.2% (Figure 2B). There was no statistical difference between the anti-SARS-CoV-2 spike cross-reactivity prevalence rates, with 2.9% in Tanzania, 4% in Zambia and 1.2% in USA (Figure 2C). Additionally, none of the cross-reactive samples from Tanzania are HIV-1 positive and only 5 out of 43 (11.6%) HIV-1 positive samples from Zambia were cross-reactive towards SARS-CoV-2. Whereas, 9 out of 56 (16%) HIV-1 negative samples from Zambia were cross-reactive towards SARS-CoV-2. Therefore, HIV-1 infected individuals seem to have lower cross-reactive response towards SARS-CoV-2. However, a larger sample size of an HIV-1 positive cohort will be needed to verify this observation.

To investigate whether anti-SARS-CoV-2 cross-reactivity correlated with past exposures to other human coronaviruses, pre-COVID-19 pandemic plasma samples that cross-reacted against SARS-CoV-2 were tested for their anti-HCoV responses. As demonstrated with a representative cross-reactive plasma sample 21854, IFA against the spike and nucleocapsid

proteins of different HCoV revealed IgG antibodies against HCoV-OC43, HKU-1, NL63 and 229E spike proteins (Figure 3). However, the same plasma sample only recognized the nucleocapsid of HCoV-NL63, suggesting that HCoV-NL63 could be the main source of antigenic exposure for this individual. When we analyzed all SARS-CoV-2 serologically cross-reactive samples, we found 100% recognized the spike proteins from all four HCoV that cause the common cold, but not that from SARS and MERS (Figure 4A). This recognition of common HCoV spike versus SARS and MERS spike proteins was statistically significant ($P < 0.0001$). Additionally, comparison of HCoV nucleocapsid recognition among all samples showed that the most commonly recognized nucleocapsid was that of HCoV-NL63, followed by HCoV-229E, at 92% and 50%, respectively (Figure 4B). This difference was statistically significant compared to recognition of the other HCoV, with P-values ranging from < 0.0001 to 0.0002 for HCoV-NL63 and P-values ranging from 0.0002 to 0.0054 for HCoV-229E (Figure 4B). Lastly, we compared how individuals from different countries responded against various HCoV nucleocapsid. Qualitatively, we found that the Zambian SARS-CoV-2 cross-reactive samples tended to recognize a wider range of HCoV compared to samples from Tanzania (Table 2). Some Zambian individuals recognized 4 up to 6 different HCoV, whereas Tanzanian individuals maximally recognized 3 different HCoV. However, the small sample size limited the statistical analysis of this difference.

Discussion

Despite the rapid spread of SARS-CoV-2 and causing nearly a million deaths worldwide to date, the SARS-CoV-2 burden in sub-Sahara Africa remains surprisingly low. This is despite a high prevalence of other diseases such as HIV-1, malaria, cancer and tuberculosis, and in

addition to insufficient health care and the impact of poverty. Coincidently, the current SARS-CoV-2 disease burden is much higher in the USA than sub-Saharan African countries. Whether this low prevalence of serological cross-reactivity to HCoV in the USA, as we report here, is directly associated with the outcomes of the USA COVID-19 pandemic remains unknown. Our data suggests that populations in sub-Sahara Africa had been pre-exposed to a spectrum of HCoVs that have provided some cross-reactivity against SARS-CoV-2 and may have limited infections or pathogenesis on the continent. In support of this hypothesis, our study detected serological cross-reactivity against SARS-CoV-2 antigens in pre-COVID-19 plasma samples from Tanzania and Zambia at levels nearly 8- and 6-fold, respectively, higher than the prevalence in samples from the USA. Additionally, by comparing the prevalence of serological cross-reactivity against SARS-CoV-2 between HIV-1 positive and negative Zambian individuals, we found that HIV-1 infection seem to lower the cross-reactive response towards SARS-CoV-2, which could be caused by a weakened immune response in HIV-1 infected individuals. However, a larger sample size of HIV-1 positive cohort will be needed to confirm this observation.

Among our study cohort, individuals reactive to SARS-CoV-2 antigens predominantly cross-reacted with SARS-CoV-2 nucleocapsid protein. Consistent with spike protein variation across coronaviruses, few individuals reacted with the SARS-CoV-2 spike protein. This reaffirmed that SARS-CoV-2 spike is a more specific target for serological testing for SARS-CoV-2 infection and humoral response to infection. Conversely, a recent study suggests that SARS-CoV-2 nucleocapsid is more sensitive than spike for early detection of SARS-CoV-2 infection (Burbelo et al., 2020) highlighting the distinction between sensitivity and specificity. Based on our analysis of pre-COVID-19 pandemic samples, we would support the notion that

detection of SARS-CoV-2 infection with nucleocapsid may generate a significant number of false positive results which could be country specify, with countries like Tanzania and Zambia potentially having a higher false positive rate than USA due to prior exposure to other coronaviruses.

To address the question of which human coronavirus was responsible for the observed cross-reactivity with SARS-CoV-2, we found that all SARS-CoV-2 cross-reactive samples strongly cross-reacted with the spike proteins from HCoV-OC43, HCoV-HKU-1, HCoV-NL63 and HCoV-229E, but not from SARS or MERS. This suggests that some immunogenic epitopes within the spike protein may be shared among all the known human coronaviruses. Additionally, the majority of our SARS-CoV-2 cross-reactive samples reacted strongly against the nucleocapsid of HCoV-NL63 and HCoV-229E, suggesting that these two HCoVs may have served as the source of antigenic exposure in sub-Saharan Africa prior to the COVID-19 pandemic. Although cross-reactivity against SARS-CoV-1 nucleocapsid as a result of exposure to human coronaviruses, such as HCoV-OC43, has been reported (Patrick et al., 2006), ours is the first study linking HCoV-NL63 and HCoV-229E to cross-reactivity against SARS-CoV-2 in the sub-Sahara Africa setting.

HCoV-NL63 and HCoV-229E are members of alphacoronavirus, whereas HCoV-OC43, HCoV-HKU-1 belongs to the same betacoronavirus as SARS-CoV-2 (Abdul-Rasool and Fielding, 2010). Additionally, HCoV-NL63 is the only other human coronavirus that uses angiotensin-converting enzyme (ACE) 2, the same receptor used by SARS and SARS-CoV-2 (Abdul-Rasool and Fielding, 2010, Hofmann et al., 2005). The epidemiology of HCoV-NL63 and HCoV-229E in adults is poorly defined. Some studies reported an 8.8% prevalence rate of HCoV-NL63 in the USA with less than 1% in UK (Esper et al., 2005, Gaunt et al., 2010). The

prevalence of HCoV-229E is unclear. Importantly, no epidemiological data exists for these two HCoVs in sub-Saharan Africa. Additionally, a recent bioinformatics study suggests that SARS-CoV-2 evolved from bat coronavirus and may have bats as a primary reservoir (Boni et al., 2020). Given the abundance wildlife, including multiple species of bats, in Africa and its often close proximity to humans, we cannot exclude the possibility of exposure to zoonotic coronaviruses that could elicit the observed cross-reactivity against SARS-CoV-2 and other human coronaviruses. Our results would suggest that infections with HCoV-NL63 or similar transmissible zoonotic agents were common in sub-Saharan Africa prior to the COVID-19 pandemic.

Lastly, the function of these SARS-CoV-2 cross-reactive antibodies and whether they provide any protection against SARS-CoV-2 infection or disease progression is still unclear and cannot be resolved with retrospective cross-sectional sampling. Since SARS-CoV-2 nucleocapsid is the major antigen that is recognized by these cross-reactive antibodies, we speculate that antibody-dependent effector mechanisms such as antibody-dependent cellular cytotoxicity could play some protective role. Our finding of SARS-CoV-2 cross-reactive antibodies in pre-COVID-19 pandemic samples mirrors and supports a recent study that showed exposure to HCoV/common cold induced SARS-CoV-2 cross-reactive T-cell responses in pre-pandemic samples (Mateus et al., 2020). Perhaps both adaptive responses may have offered some protection against COVID-19 pathogenesis, if not SARS-CoV-2 infection. A limitation of our study is that there were no peripheral blood mononuclear cells collected prior to the COVID-19 pandemic for analysis of potential cross-reactive T-cell response. Thus, a larger sample size and more in-depth longitudinal analysis of the function of these cross-reactive antibodies, as well as cross-reactive T-cell response will be needed in future studies.

Author contributions

FYT, SJL, PBP and AAC performed IFA. PBP and AAC performed HIV serological testing. ON, PJ, JRN, JM and JTW collected all plasma samples. FYT wrote the manuscript. CW supervised all aspect of the study. All authors reviewed and approved the manuscript.

Conflict of interest

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

Acknowledgement

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Table 1: Study cohort and sampling time period

Country	Sample size	HIV-1 positive (%)	Sampling time period
Tanzania	105	7 (6.7%)	March to May 2019
Zambia	99	43 (43.4%)	2017 to early 2019
USA	85	0 (0%)	2005, 2007 and 2009

Table 2: Individual cross-reactive responses against the nucleocapsid protein of SARS, MERS, HCoV-OC43, HCoV-HKU-1, HCoV-NL63 and HCoV-229E.

Country	Sample ID	SARS	MERS	OC43	HKU-1	NL63	229E
Tanzania	**21850**	-	-	-	-	+	-
Tanzania	**21854**	-	-	-	-	+	-
Tanzania	**21868**	-	-	-	-	+	+
Tanzania	**21872**	-	-	-	-	+	+
Tanzania	**21873**	-	-	-	-	+	-
Tanzania	**21928**	-	-	-	-	+	-
Tanzania	**21933**	-	-	-	-	+	-
Tanzania	**211141**	+	-	-	-	+	+
Tanzania	**211145**	-	-	-	-	+	+
Tanzania	**211157**	-	-	-	+	+	+
Tanzania	**211176**	+	-	-	-	+	-
Tanzania	**211177**	-	-	-	-	+	-
Tanzania	**211181**	-	-	-	-	+	+
Tanzania	**211182**	-	-	-	-	+	-
Tanzania	**211185**	-	-	-	+	+	+
Tanzania	**211188**	-	-	-	-	+	+
Tanzania	**211192**	-	+	-	-	+	+
Tanzania	**211203**	-	-	-	-	+	+
Tanzania	**211205**	-	-	-	-	+	+
Tanzania	**211210**	-	-	-	-	+	+
Zambia	**C3076**	+	-	-	-	+	-
Zambia	**C3082**	-	-	-	-	+	-
Zambia	**C3154**	-	-	-	-	+	-
Zambia	**C3155**	+	-	+	+	+	+
Zambia	**C3156**	-	-	-	-	+	+
Zambia	**C3163**	+	-	+	-	+	+
Zambia	**C3166**	-	+	-	-	+	-

Zambia	C3182	+	-	+	+	+	+
Zambia	C3187	-	-	-	-	-	-
Zambia	C3197	-	-	-	-	+	-
Zambia	C3202	+	+	+	+	+	+
Zambia	C3204	+	+	-	-	-	-
Zambia	N044	+	-	-	-	+	-
Zambia	N216	+	-	-	-	+	-
USA	KC-34	-	-	-	-	+	+
USA	KC-65	-	-	-	-	+	+

Figure 1. Immunofluorescence assay (IFA) against either mock, SARS-CoV-2 spike or nucleocapsid expressing cells. Representative pictures of IFA with negative control plasma, COVID-19 convalescence plasma (positive control) and pre-COVID-19 pandemic cross-reactive plasma samples 21928 and 21933. Sample 21928 displayed cross-reactivity against SARS-CoV-2 spike, but not its respective mock and SARS-CoV-2 nucleocapsid. Sample 21933 displayed cross-reactivity against SARS-CoV-2 nucleocapsid, but not its respective mock and SARS-CoV-2 spike. White arrows indicate positive cells. Scale bar represent 50 µm.

Figure 2. Percent prevalence of serological cross-reactivity against SARS-CoV-2 among Tanzania, Zambia and USA. (A) Combined serological cross-reactivity against SARS-CoV-2 spike and nucleocapsid. (B) Serological cross-reactivity against SARS-CoV-2 nucleocapsid. (C) Serological cross-reactivity against SARS-CoV-2 spike.

Figure 3. Immunofluorescence assay (IFA) against SARS, MERS, HCoV-OC43, HCoV-HKU-1, HCoV-NL63 and HCoV-229E spike or nucleocapsid expressing cells. Representative pictures of IFA with pre-COVID-19 pandemic cross-reactive plasma samples 21854. Sample 21854 strongly recognized the spike protein of HCoV-OC43, HCoV-HKU-1, HCoV-NL63 and HCoV-229E, but not SARS and MERS. Sample 21854 only recognized the nucleocapsid of HCoV-

NL63 and not the other human coronaviruses. White arrows indicate positive cells. Scale bar represent 50 µm.

Figure 4. Percent prevalence of serological cross-reactivity against SARS, MERS, HCoV-OC43, HCoV-HKU-1, HCoV-NL63 and HCoV-229E. (A) Spike. (B) Nucleocapsid.

Figure 1

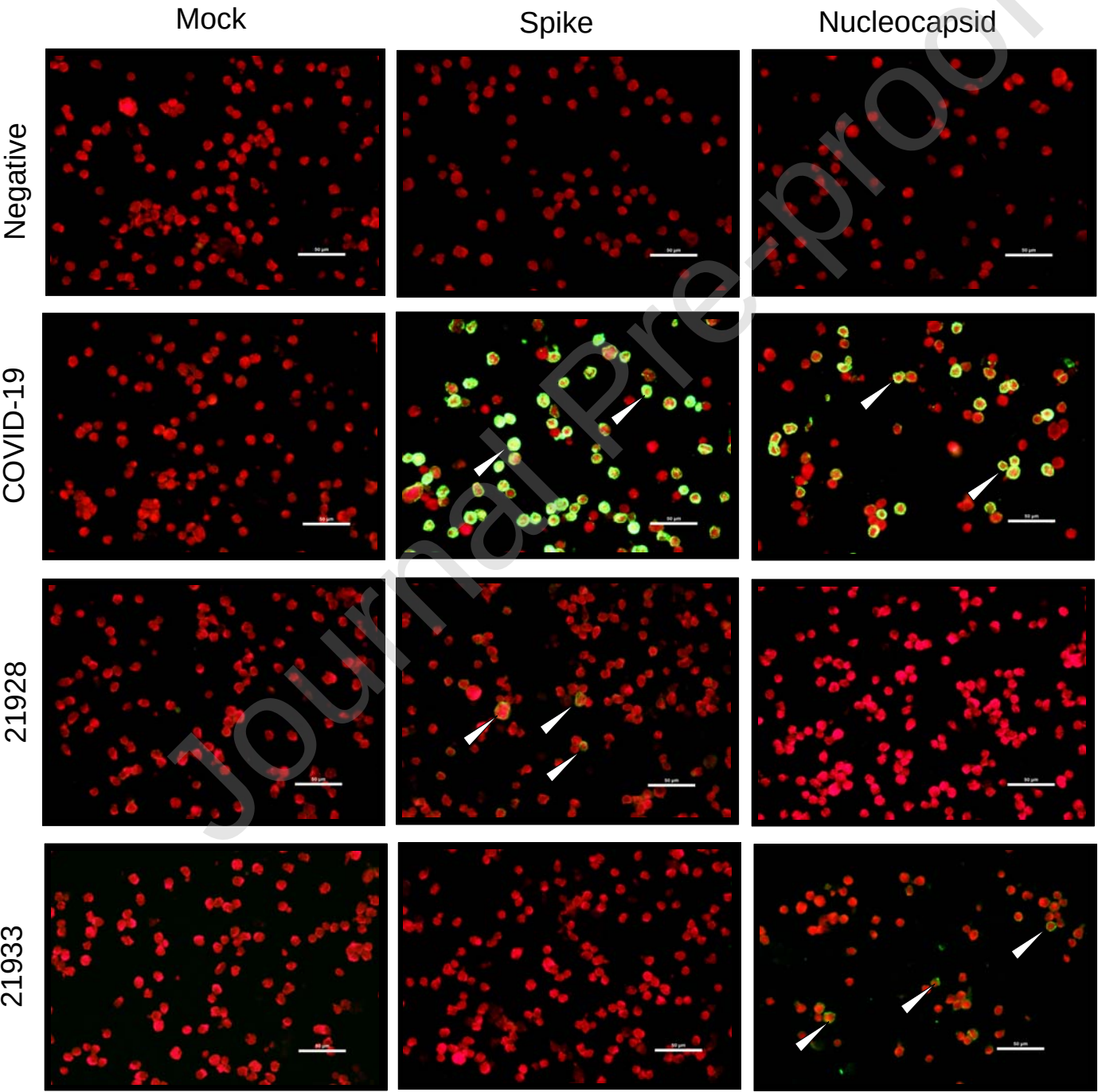
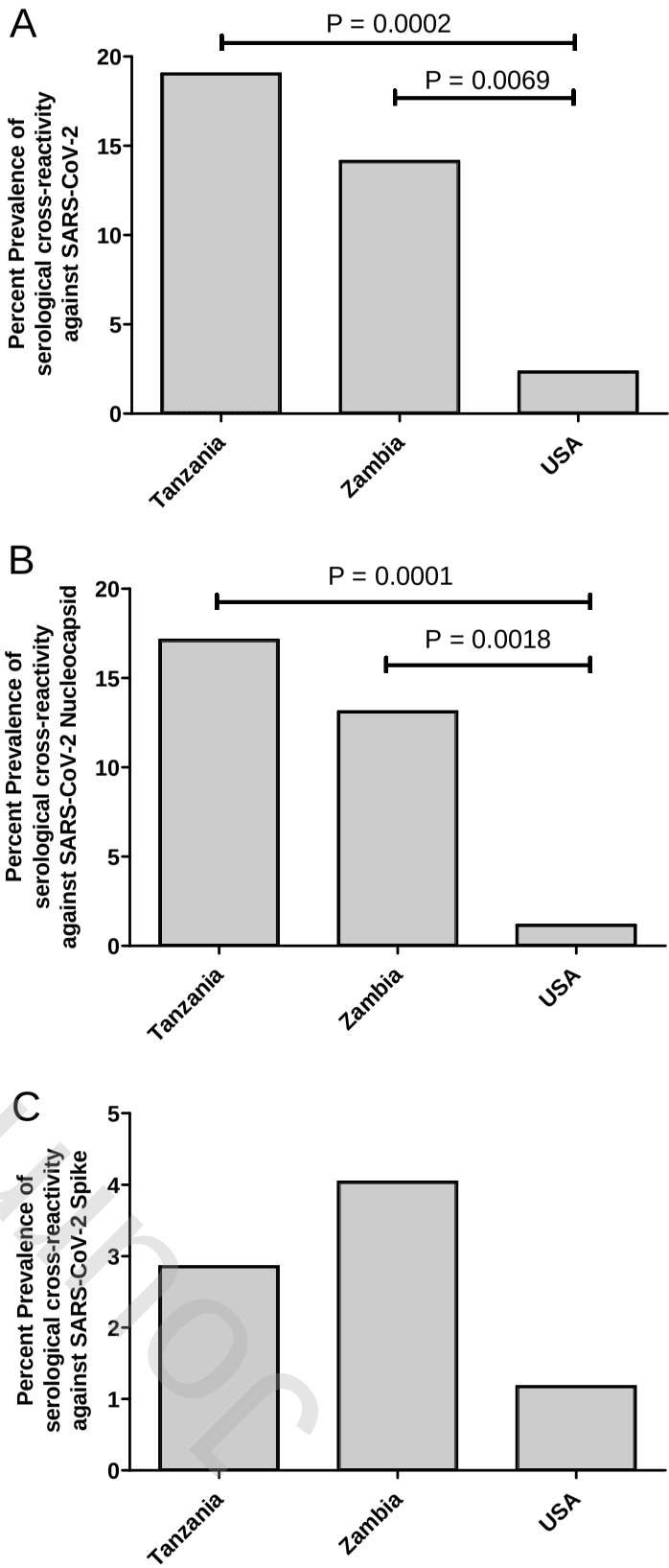
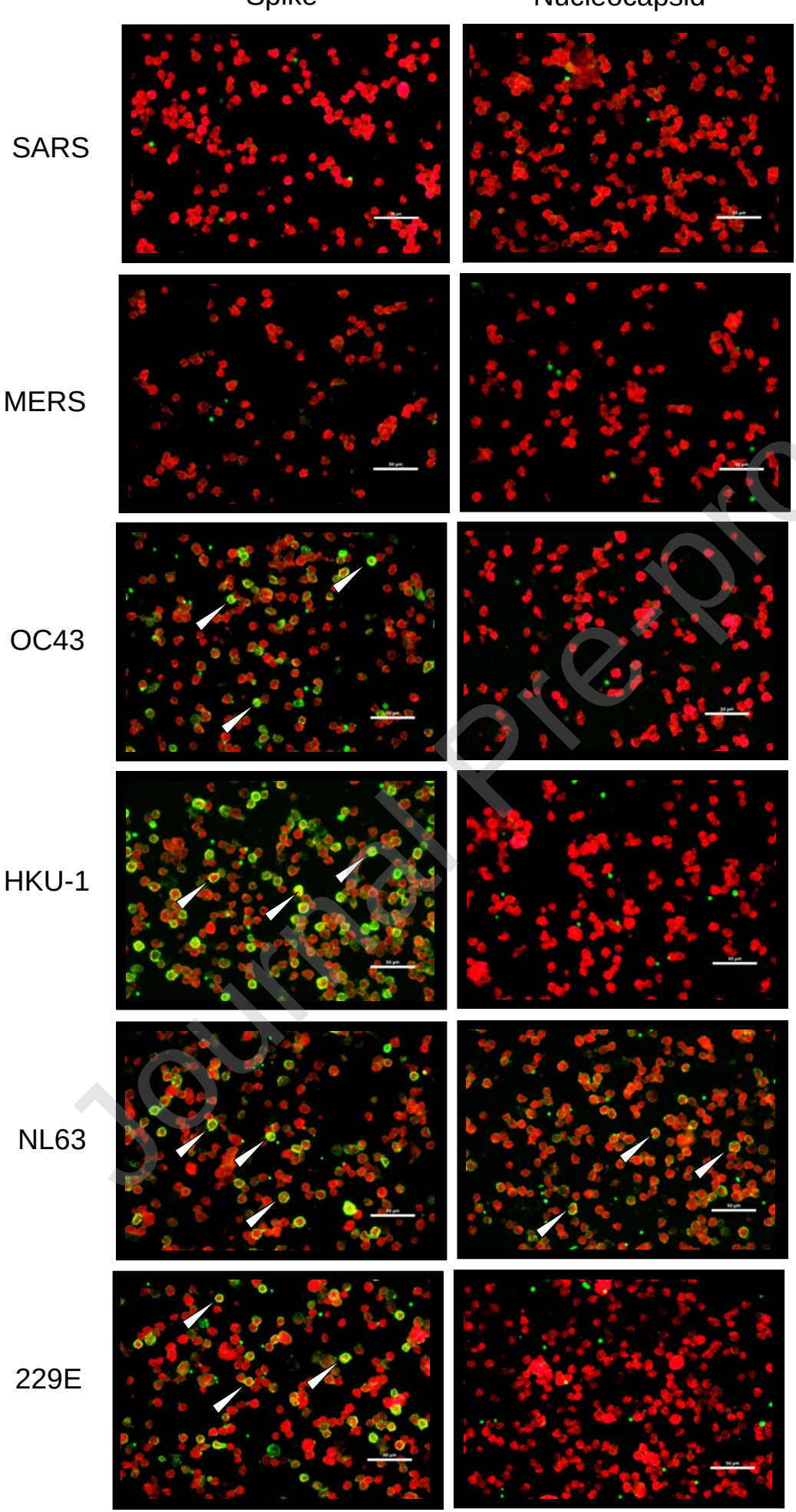
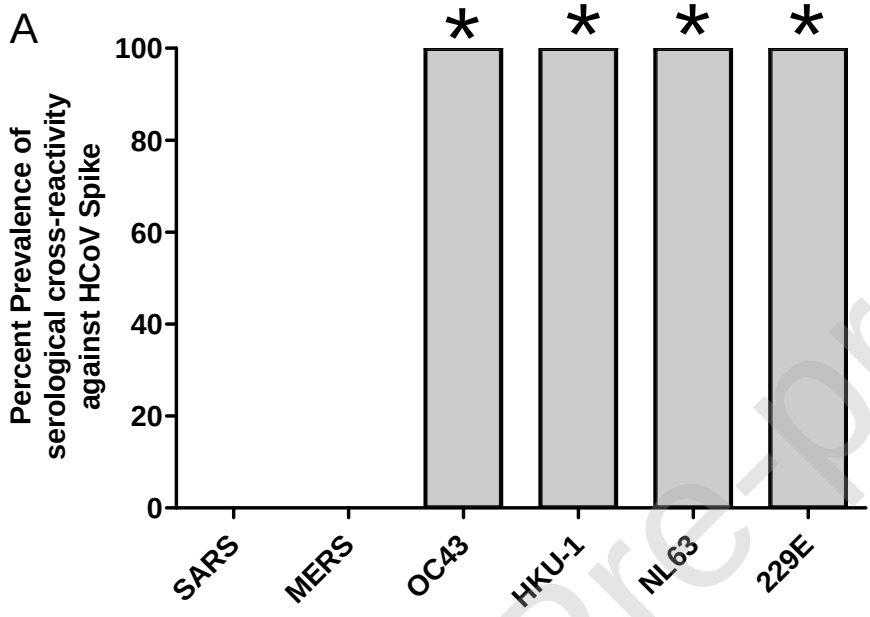
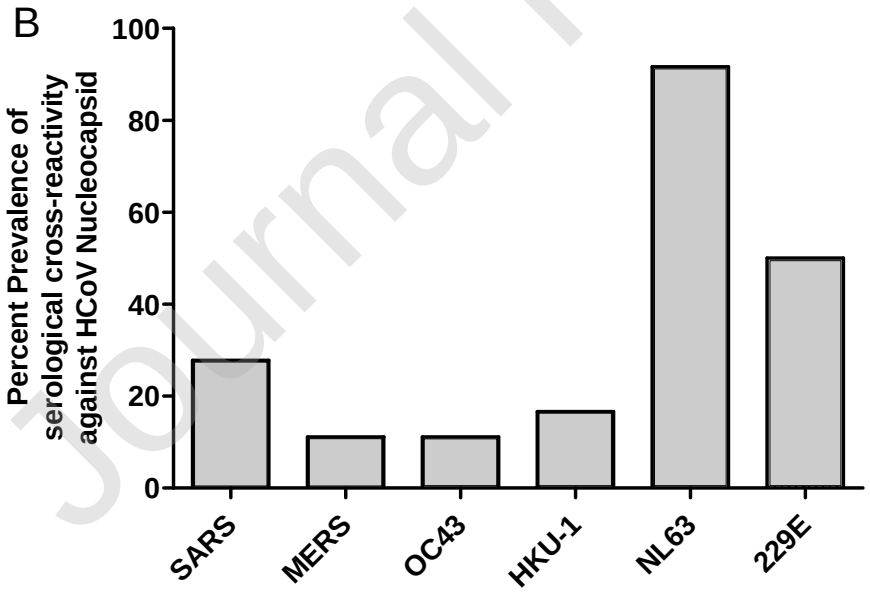


Figure 2


A
P = 0.0002
P = 0.0069
Percent Prevalence of serological cross-reactivity against SARS-CoV-2
20
15
10
5
0
Tanzania
Zambia
USA
B
P = 0.0001
P = 0.0018
Percent Prevalence of serological cross-reactivity against SARS-CoV-2 Nucleocapsid
20
15
10
5
0
Tanzania
Zambia
USA
C
Percent Prevalence of serological cross-reactivity against SARS-CoV-2 Spike
5
4
3
2
1
0
Tanzania
Zambia
USA







P-values table

	SARS	MERS	OC43	HKU-1	NL63	229E
SARS	-	ns	ns	ns	<0.0001	ns
MERS	ns	-	ns	ns	<0.0001	0.0007
OC43	ns	ns	-	ns	<0.0001	0.0007
HKU-1	ns	ns	ns	-	<0.0001	0.0054
NL63	<0.0001	<0.0001	<0.0001	<0.0001	-	0.0002
229E	ns	0.0007	0.0007	0.0054	0.0002	-

"ns" denotes non-significant