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Original Research

Construction of pseudotyped human coronaviruses and detection of pre-existing antibodies in the human population

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ABSTRACT

In order to clarify the pre-exist immunity background of different human coronaviruses (HCoV), this study investigated the positive rate of spike (S) protein antibodies of HCoV, including HCoV- severe acute respiratory syndrome (SARS) −associated coronavirus (SARS-CoV-1), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Middle East respiratory syndrome coronavirus (MERS-CoV), HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43, before and after the Coronavirus Disease 2019 (COVID-19) outbreak. We utilized pseudotyped virus-based neutralization assays (PBNA) or enzyme-linked immunosorbent assays (ELISA) to detect antibody levels against HCoV in serum samples collected in 2009–2010 and 2023. The PBNA results showed that neutralizing antibodies against SARS-CoV-1 and the MERS-CoV were negative. In the serum samples from 2009 to 2010, neutralizing antibodies against SARS-CoV-2 (D614G) were negative, whereas in the serum samples from 2023, 73 samples (73 %) showed neutralizing reactions with the SARS-CoV-2 D614G strain, 96 samples (96 %) with the BA.5 strain, and 91 samples (91 %) with the BF.7 strain. Among pre-COVID-19 samples, 33 % (33/100) showed neutralizing reactions with HCoV-229E and 63 % (63/100) with HCoV-NL63. Among post-COVID-19 samples, 50 % (50/100) showed neutralizing reactions with HCoV-229E and 49 % (49/100) with HCoV-NL63. Due to the different receptors of alpha coronavirus genus compared to other beta coronavirus genus, neutralizing antibodies against HCoV-OC43 and HCoV-HKU1 virus cannot be detected by constructing corresponding pseudotyped virus. Binding antibodies against HCoV-OC43 and HCoV-HKU1 virus were detected using ELISA. The results revealed that among pre-COVID-19 samples, 83 % (83/100) and 45 % (45/100) had binding activity with HCoV-OC43 and HCoV-HKU1, respectively. Among post-COVID-19 samples, 100 % (100/100) and 81 % (81/100) had binding activity with HCoV-OC43 and HCoV-HKU1, respectively.

1. Introduction

Seven human-infecting coronaviruses have been identified: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV-1), Middle East respiratory syndrome coronavirus (MERS-CoV), and the human coronaviruses (HCoVs) including HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43. Among them, HCoV-229E and HCoV-NL63 belong to the alpha coronavirus genus, whereas the remaining five belong to the beta coronavirus genus [1].

HCoV spread primarily through respiratory droplets, aerosols, and direct contact with surfaces that are contaminated with the virus. Fecal–oral transmission was also reported during the SARS epidemic [2]. Among HCoVs, SARS-CoV-1, SARS-CoV-2, and MERS-CoV can cause severe respiratory syndromes, whereas the remaining four coronaviruses are common respiratory pathogens that exhibit some seasonality and cause mild respiratory infections and the common cold. The symptoms of infection are relatively mild and account for 15 %–30 % of common cold cases [3,4]. These coronaviruses are also associated with gastrointestinal diseases and pneumonia. However, in infants, older adults, and immunocompromised individuals, these viruses can

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HIGHLIGHTS

Scientific question

This study aims to explore the prevalence of seven human coronaviruses before and after the outbreak of coronavirus disease 2019 (COVID-19), as well as to assess the levels of antibodies against these coronaviruses in the population.

Evidence before this study

Serological studies on severe acute respiratory syndrome (SARS-CoV-1), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and Middle East respiratory syndrome coronavirus (MERS-CoV) are abundant. However, there is a relative lack of seroepidemiological research on human coronavirus-229E (HCoV-229E), HCoV-NL63, HCoV-HKU1, and HCoV-OC43. Furthermore, the highly pathogenic nature of SARS-CoV-1, SARS-CoV-2, and MERS-CoV, along with the challenges in acquiring various viral strains, have impeded comprehensive studies in this domain.

New findings

We established a pseudotyped virus-basedvirus-based assay to evaluate neutralization antibody levels. A comprehensive evaluation of prevalence and antibody levels against the seven human coronaviruses has been performed on populations before and after the COVID-19 outbreak.

Significance of the study

A pseudotyped virus based assay to evaluate neutralization antibody levels was established. Seroepidemiological research of all seven human coronaviruses was performed and compared in the same population.

lead to more severe clinical symptoms [5,6]. Additionally, relevant studies indicate that these four seasonal coronaviruses may cause natural reinfections [7]. Coronaviruses infect cells mainly through the binding of spike (S) protein to cell surface receptors. S protein binding antibodies and neutralizing antibodies are protective antibodies, the level of which reflects pre-existing immunity against different coronaviruses. Understanding the positive rate of coronavirus S protein antibodies in the population is crucial and supportive for coronavirus-related research and vaccine development. However, a systemic survey of serology antibody levels of four seasonal coronaviruses was lacking. So, in present study, we investigated the positive rate of S protein antibodies of all seven HCoV, which provided information of the immune background in the population.

2. Materials and methods

2.1. Materials

2.1.1. Plasmid

The S protein genes of seven HCoV, including SARS-CoV-1 (GenBank: AY278491.2), SARS-CoV-2 with 21aa deletion on C-terminal (D614G: GenBank: QRG21497.1, BA.5: GenBank: WJF30936.1. BF.7 introduces an additional R346T mutation on the basis of BA.5.), MERS-CoV (GenBank: QJX19878.1), HCoV-NL63 (GenBank: BDG58142.1), HCoV-229E (GenBank: UJH59859.1), HCoV-HKU1 (GenBank: WKE35756.1) and HCoV-OC43 (GenBank: BDG58142.1), were codon-optimized and cloned into the pcDNA3.1 vector.

2.1.2. Serum

One hundred serum samples from Guangxi Zhuang Autonomous Region were collected in 2009–2010 and stored below −20 ℃ by the National Institutes for Food and Drug Control. In 2023, an additional 100 serum samples from Shandong Province were collected by Taibang Biologic Group (Shandong Province, China). All serum samples were collected with informed consent for pathogen marker detection.

2.1.3. Cell lines

The cell lines in the experiment included human embryonic kidney cells contains the SV40 large T antigen (293 T), 293 T cells that stably expressed the angiotensin-converting enzyme 2 (ACE2) receptor and furin genes (293 T-AF), human hepatocellular carcinoma cells (Huh-7), African green monkey kidney cells (Vero), human embryonic kidney cells with high expression of β galactoside α-2,6-sialyltransferase (293 T-α2-6), human lung cancer cells (A549), baby hamster kidney cells (BHK21), Chinese hamster ovary cells, human embryonic kidney cells fast growing variant contains the SV40 large T antigen (293FT), human cervical cancer cells (HeLa), human hepatocellular carcinoma cells (HepG2), canine thymus cells (Cf2TH), Rhesus monkey kidney cells (LLC-MK2), bovine kidney cells (MDBK), canine kidney cells (MDCK), human lung fibroblast cells (MRC5), and mouse embryo fibroblast cells (NIH-3 T3).

2.1.4. Enzyme-linked immunosorbent assay (ELISA) kits

The ELISA kits were prepared by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd (BeiJing City, China). S proteins of HKU1 and OC43 were bought from the other company − SinoBiological (Cataloge number: 40606-V08B, 40607-V08B). S proteins of HKU1 and OC43 were diluted to 250 ng/mL in a buffer at a 4,000-fold dilution as antigens. Each well received 100 μL of the diluted solution, followed by an incubation at 37 ˚C for 2 h and overnight incubation at 4 ˚C. After the night, the blocking solution was discarded, and a closure solution was applied for 2 h, followed by discarding the closure solution and allowing it to dry.

The cut-off value of 0.15 was established by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. through relevant research.

2.2. Preparation of human coronavirus pseudotyped virus

Pseudotyped virus was generated as previously described. The 293 T cells were cultured in a T75 flask until they reached a density of 80 % – 90 %. The culture medium was discarded, and 15 mL of dulbecco's modified eagle medium (DMEM) complete medium containing 7.0×10^4 tissue culture infective dose (TCID) 50 / mL of *Vesicular stomatitis* virus (VSV) pseudotyped virus was added. Additionally, 30 μg of pcDNA3.1 (+) expressing the coronavirus S protein was transfected using Lipofectamine 3,000 reagent (Thermo Fisher Scientific, Massachusetts, United States). After incubation at room temperature, the sample from Tube A was added to the 293 T cells and incubated in a 5 % CO_2 atmosphere at 37 ˚C. After 4–6 h, the culture medium was discarded, and the cells were washed three times with 10 mL of phosphate buffered saline (PBS) containing 1 % fetal bovine serum (FBS). Then, 15 mL of fresh DMEM medium was added for further cultivation.

After 24 h, the supernatant was collected and stored at 4 ˚C. Another 15 mL of DMEM medium was added to the 293 T cells, and the supernatant was collected after an additional 24 h of cultivation. The two collected supernatants were mixed, centrifuged at 1,000 rpm for 10 min, filtered through a 0.45 μm filter, and stored at − 80 ˚C.

2.3. Cell tropism test

A total of 17 cell lines were digested using trypsin, and 30,000/100 μL cells were added to each well of the 96-well plate.

After incubation in a 37 °C incubator in a 5 % CO_2 atmosphere for 24 h, chemiluminescence detection was performed. Luciferase substrate (100 μL, PerkinElmer, Massachusetts, USA) was added to each well, and the wells were incubated at room temperature for 2 min and transferred to the detection whiteboard. Measurement was then performed using a luminometer (PerkinElmer). Each group contained three replicates.

2.4. Pseudotyped virus based neutralization assay (PBNA)

High-pressure sterilized water (250 μL) was added to the surrounding 36 wells of a 96-well plate, and the edges were sealed. The serum samples were diluted 30 times using DMEM complete medium. Sequentially, 150 μL was added to wells B4–B11, and 100 μL medium was added to the remaining wells. Fifty microliters of liquid were transferred from wells B4–B11 to wells C4–C11, and a three-fold serial dilution was performed for all wells. Subsequently, 50 μL of pseudotyped virus suspension was added to each well in columns 3–11 (with B3–F3 as the virus control). After 1 h, 100 μL of 293 T-AF cells was added to each well (MERS-CoV and HCoV-229E pseudotyped viruses were added to Huh-7 cells). The wells were incubated at 37 °C for 24 h in a CO_2 incubator, the liquid was discarded, 100 μL of fluorescence detection reagent was added, and the wells were placed in the dark at room temperature for 2 min. Finally, the PerkinElmer EnSight multifunctional imaging plate reader was used to read relative light units and calculate the neutralization inhibition rate [8].

2.5. ELISA

Serum samples were diluted 100-fold. Each well was loaded with 100 μL of the diluted serum, followed by a 30-minute incubation at 37 °C. Afterward, five washes were performed, and each well received 100 μL of enzyme-labeled reagent, followed by a 30-minute incubation at 37 °C. Another five washes were carried out, and then 50 μL each of color development solutions A and B were added to each well. After a 15-minute light-protected color development, 50 μL of stop solution was added to each well.

Finally, the ELISA reader was employed with dual wavelengths set at 450 nm / 620 nm for detection.

2.6. Statistical analysis

All data were analyzed and graphs were generated using GraphPad Prism 6.0 software (GraphPad, San Diego, CA, USA). *P*-values of < 0.05 were considered statistically significant.

3. Results

3.1. Selection of infected cells of seven HCoV

Because SARS-CoV, MERS-CoV, and SARS-CoV-2 are highly pathogenic, we utilized the VSV system of pseudotyped viruses that express the envelope proteins of the studied viruses. We infected VSVdeltaG*G with plasmids that encoded the envelope proteins of coronaviruses and collected the supernatant to obtain the pseudotyped virus. We assessed the tropism of the pseudotyped virus for various cell types: 293 T-AF, Huh-7, Vero, 293 T-α2-6, A549, BHK21, 293FT, HeLa, HepG2, Cf2TH, LLC-MK2, MDBK, MDCK, MRC5, and NIHH-3 T3. SARS-CoV, SARS-CoV-2, and NL63-CoV infected 293 T-AF, Huh-7, Vero, 293 T, 293FT, 293 T-α2-6, and LLC-MK2; the highest titer was observed with 293 T-AF. NL63-CoV demonstrated titers to 293 T-AF, and MERS-CoV showed titers to Huh-7 and LLC-MK2 with higher titers to Huh-7. Using aminopeptidase N as the receptor, 229E-CoV showed titers to Huh-7. HKU1 and OC43 were not detected with infectious titers in any of the cell lines tested (Fig. 1A-G). In this study, we used the 293 T-AF cell line for pseudotyped viruses including SARS-CoV, SARS-CoV-2, and HCoV-NL63 that were capable of infecting cells, and the Huh-7 cell line was used for MERS-CoV and HCoV-229E, for which sensitive cell lines were identified. To further clarify the background of the pseudoviruses, we infected 293 T with VSV without transfection of plasmids expressing envelope proteins. Supernatant was collected as non-envelop VSV control. Titration was perform using either non-enveloped VSV envelope and enveloped VSV pseudovirus in parallel in sensitive cell lines. Relative light unit (RLU) of enveloped pseudovirus were at least 100 times than RLU of non-enveloped VSV in all successfully packaged virus. For the unsuccessfully packaged ones, OC43 and HKU1, RLU of those were only 27 and 45 times over control (Fig. 1H).

3.2. Serum antibody level detection

The neutralizing antibody median effect concentration (EC_{50}) was determined by serum neutralization. For HCov-OC43 and HCov-HKU1, for which sensitive cell lines were not identified, binding antibodies were measured using the ELISA method. The results of antibody level detection for 200 serum samples are presented in Table 1. In this experiment, the cut-off value for SARS-CoV-1, SARS-CoV-2, MERS-CoV, and HCoV-229E was defined as 30, while the cut-off value for HCoV-NL63 was defined as 183. In serum samples collected from Guangxi Zhuang Autonomous Region in the years 2009 and 2010, neutralizing antibodies against SARS-CoV, SARS-CoV-2, and MERS-CoV were all negative. Among these samples, 63 % (63 / 100) and 33 % (33 / 100) exhibited neutralizing reactions with HCoV-NL63 and HCoV-229E, respectively (Fig. 2). Additionally, 83 % (83 / 100) and 45 % (45 / 100) samples showed binding reactions with HCoV-OC43 and HCov-HKU1, respectively (Fig. 3). The average neutralizing antibody titers against HCoV-NL63 and HCoV-229E in the population samples from Guangxi Zhuang Autonomous Region were 393 and 84, respectively.

Among the serum samples collected in Shandong Province in 2023, neutralizing antibodies against SARS-CoV and MERS-CoV were negative. Respectively, 73 % (73 / 100), 96 % (96 / 100), and 91 % (91 / 100) samples exhibited neutralizing reactions with the D614G, BA.5, and BF.7 strains (Fig. 2). Forty-nine percent (49 / 100) and 50 % (50 / 100) samples showed neutralizing reactions with HCoV-NL63 and HCoV-229E, respectively (Fig. 2). Additionally, 100 % (100 / 100) and 81 % (81 / 100) samples exhibited binding reactions with HCoV-OC4 and HCov-HKU1, respectively (Fig. 3). The average neutralizing antibody titers against D614G, BA.5, and BF.7 strains, HCoV-NL63 and HCoV-229E in the population samples from Shandong Province were 167, 481, 257, 424, and 80, respectively.

4. Discussion

We utilized pseudotyped viruses in this study given their structural similarity, particularly in the S protein, to live viruses that allow them to mimic the infection process without replication. This characteristic reduces the biosafety risk associated with live viruses. Previous research from us and others both showed a strong correlation between the results obtained from pseudotyped virus assays and those from live virus assays [9–14]. Therefore, in this study, we prepared seven pseudotyped viruses, five of which could infect cells and two of which did not show titers to cells. SARS-CoV, SARS-CoV-2, and HCoV-NL63 all utilize ACE2 as a receptor. 293 T-AF cells which were genetically modified 293 T cells that stably expressed the human ACE2 receptor and furin gene were used. SARS-CoV and SARS-CoV-2 demonstrated titers to 293 T-AF, Huh-7, Vero, 293 T, 293FT, 293 T-α2-6, and LLC-MK2, with the highest titer observed with 293 T-AF. This finding was consistent with our expected results. HCoV-NL63 showed titers to 293 T-AF. HCoV-MERS utilizes dipeptidyl peptidase-4 as a receptor. Although

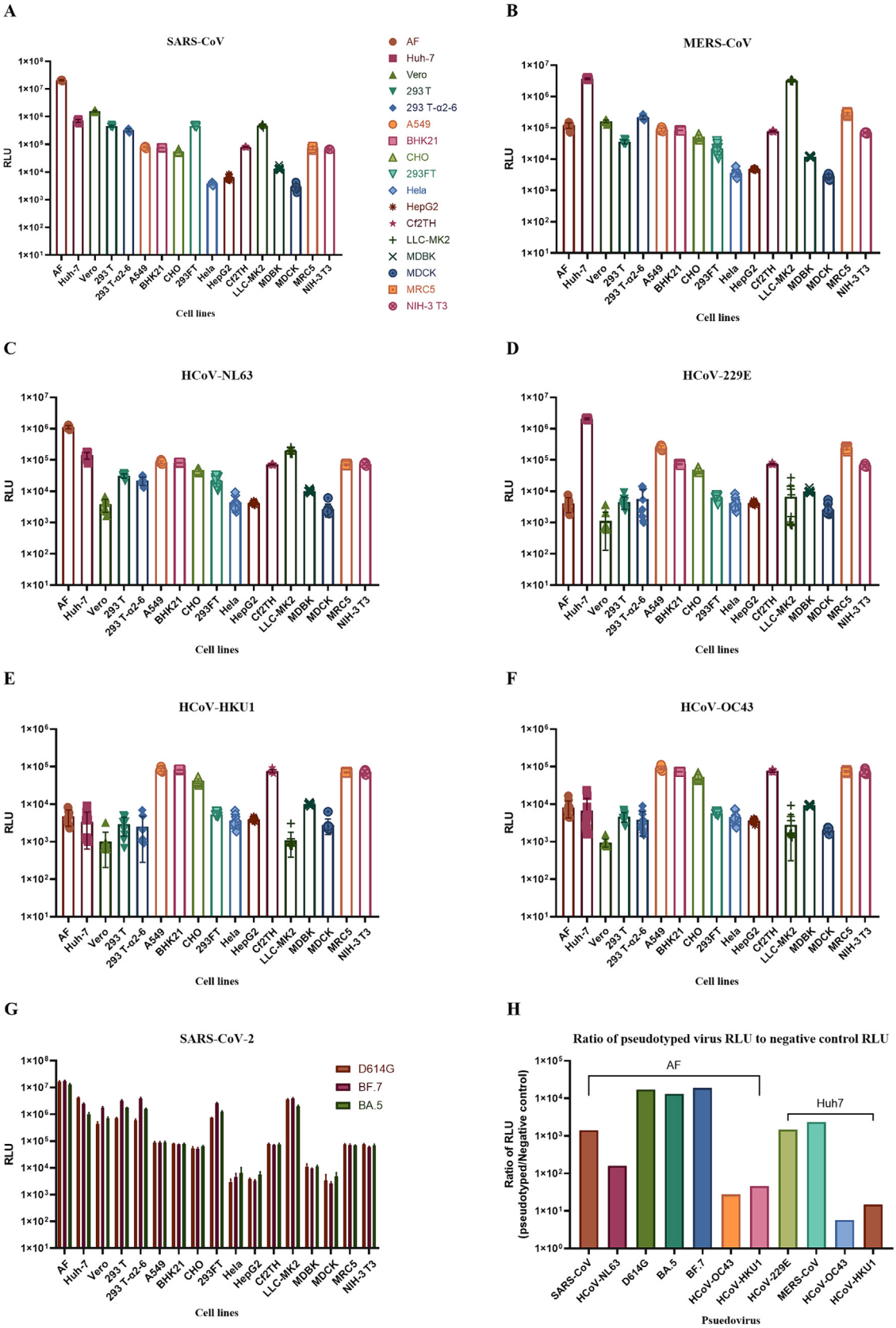

Fig. 1. The infectivity of pseudotyped viruses of 7 coronaviruses in 17 cell lines (A-G) and the ratio of pseudotyped virus RLU to negative control RLU (H). Abbreviations: AF, 293 T cells that stably expressed the angiotensin-converting enzyme 2 receptor and furin genes; Huh-7, human hepatocellular carcinoma cells; Vero, African green monkey kidney cells; 293 T, human embryonic kidney cells contains the SV40 large T antigen; 293 T-α2-6, human embryonic kidney cells with high expression of β galactoside α-2,6-sialyltransferase; A549, human lung cancer cells; BHK21, baby hamster kidney cells; CHO, Chinese hamster ovary cells; 293FT, human embryonic kidney cells fast growing variant contains the SV40 large T antigen; HeLa, human cervical cancer cells; HepG2, human hepatocellular carcinoma cells; Cf2TH, canine thymus cells; LLC-MK2, rhesus monkey kidney cells; MDBK, bovine kidney cells; MDCK, canine kidney cells; MRC5, human lung fibroblast cells; NIH-3 T3, mouse embryo fibroblast cells; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SARS-CoV-1, SARS-associated coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; HCoVs, the human coronaviruses; RLU, relative light unit.

Table 1
Proportion of samples that exhibited neutralizing (binding) reactions.

Samples	Proportion (%)	
	2009–2010 (Guangxi Zhuang Autonomous Region)	2023 (Shandong Province)
Anti-SARS	0	0
Anti-MERS	0	0
Anti-D614G	0	73
Anti-BA.5	0	96
Anti-BF.7	0	91
Anti-NL63	63	49
Anti-229E	33	50
*Anti-OC43	83	100
*Anti-HKU1	45	81

* The binding antibody results were detected using enzyme linked immunosorbent assay. Abbreviations: SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome.

HCoV-OC43 can be cultured in BHK21 and TMPRSS2 is a functional receptor for human coronavirus HKU1 [15], we did not find a sensitive cell line for the pseudotype of these two viruses. The two possible explanations are that pseudotyped viruses were not successfully packaged or sensitive cell lines were not identified. Therefore, in neutralization experiments, we chose 293 T-AF cells for SARS-CoV, SARS-CoV-2, and HCoV-NL63 and Huh-7 cells for HCoV-MERS and HCoV-229E. We performed the ELISA for antibody detection against HCoV-HKU1 and HCoV-OC43 in the human population.

In this study, either pseudotyped virus-based neutralization assays or ELISAs were performed to assess the antibody levels against seven HCoV S protein in serum samples. Initially emerging in Foshan, China in 2002, SARS-CoV caused SARS and was associated with a peak mortality rate of 10 %. By 2003, SARS had become a global infection and resulted in a SARS epidemic. By July 2003, a total of 8,096 cases had been reported. No further infections were subsequently reported, and the SARS epidemic was declared over [16–19]. In contrast, MERS-CoV was first identified in Saudi Arabia in 2012 and caused severe MERS, which is primarily characterized by lower respiratory tract symptoms such as fever, cough, respiratory distress, and pneumonia. MERS can progress to acute respiratory distress syndrome and multiple organ failure. Despite its sporadic low-efficiency transmission, MERS-CoV is associated with a mortality rate of 35 %, making it the most fatal human coronavirus [17,19–21]. Minimal evidence is currently available of SARS-CoV and MERS-CoV infections in the Chinese population. Our detection results align with this finding: no antibodies against S protein of SARS-CoV and MERS-CoV were detected in our serum samples.

SARS-CoV-2 triggered a global pandemic in this century. Patients who are infected with SARS-CoV-2 typically present with pneumonia accompanied by related symptoms of which fever and cough are the most common. As of December 2022, SARS-CoV-2 has caused over 641 million infections and has resulted in more than 6.6 million deaths. Despite its high homology with SARS-CoV-1, SARS-CoV-2 has a low fatality rate. The original strain of SARS-CoV-2 and subsequent variants often cause asymptomatic or mild infections; however, the virus has a high transmission rate and spreads rapidly, leading to severe complications in a significant number of patients worldwide [22–24]. SARS-CoV-2 has a high and continuous mutation rate. The predominant strains at the end of 2022 were BA.5 and BF.7. Therefore, we chose the original strain that harbored the D614G mutation and the prevalent strains BA.5 and BF.7 to detect antibody levels of SARS-CoV-2 S protein in serum samples from Guangxi Zhuang Autonomous Region and Shandong Province. The antibody levels against D614G S protein primarily reflected the immune response after vaccination, whereas the antibody levels against BA.5 and BF.7 S protein mainly reflected the natural infection rate. The results indicated that all serum samples from Guangxi Zhuang Autonomous Region were negative for novel coronavirus S protein antibodies. In contrast, among the serum samples from Shandong collected in 2023, the positive rates of antibodies to BA.5 and BF.7 S protein were above 90 %, with only four serum samples lacking antibodies against BA.5 and BF.7 S protein. This finding suggests that most of the Chinese population has been infected with SARS-CoV-2 and aligns with epidemiological findings, supporting the representativeness of our serum samples to the population. For the determination of positive thresholds for SARS-CoV, SARS-CoV-2, and HCoV-MERS S protein, serum samples that had median effective dose (ED_{50}) > 30 were defined as positive, whereas serum samples that had ED_{50} < 30 were considered as having ED_{50} = 15 (negative serum) in the preliminary phase of this study [9,25].

Overall, 15 %–30 % of common colds are caused by HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1. Therefore, most individuals have been exposed to these viruses and consequently carry antibod-

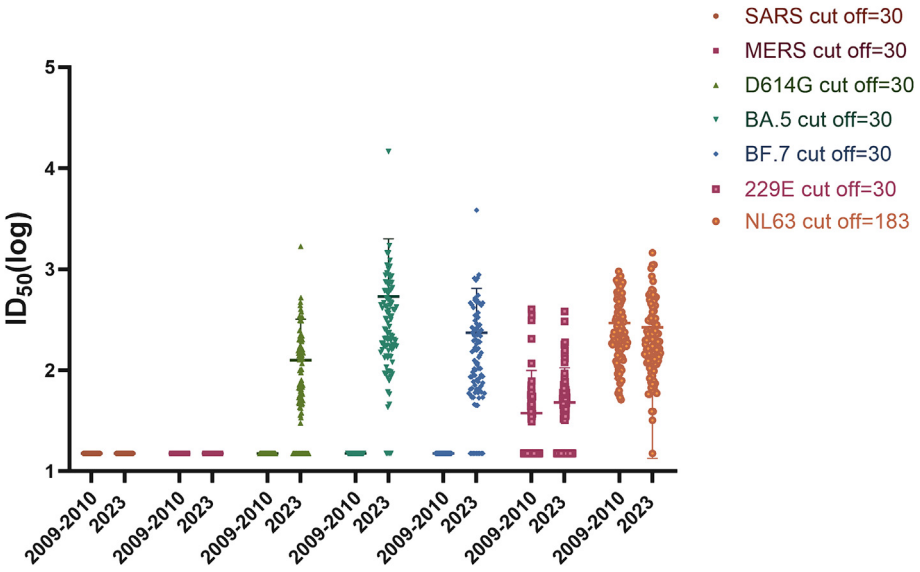

Fig. 2. SARS, MERS, D614G, BA.5, BF.7, 229E, and NL63 coronavirus spike (S) protein antibody levels in populations in Guangxi Zhuang Autonomous Region (2009–2010) and Shandong Province (2023). Abbreviations: ID_{50}, median infective dose; SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome.

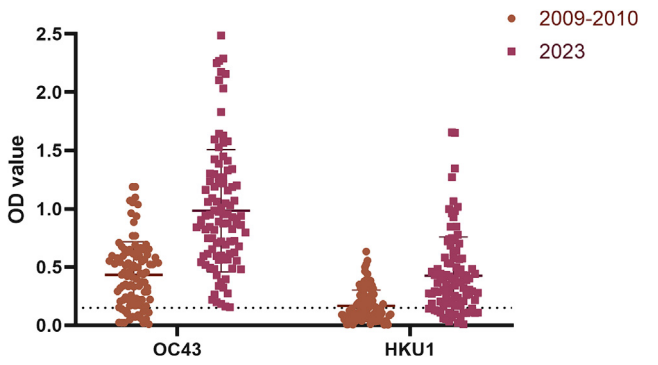

Fig. 3. Optical density (OD) levels of OC43 and HKU1 coronavirus spike (S) protein antibody in populations in Guangxi Zhuang Autonomous Region (2009–2010) and Shandong Province (2023).

ies against them [6]. Research indicates that newborns also carry maternal antibodies against HCoV-229E and HCoV-NL63 [26]. Finding human serum samples without these antibodies is extremely challenging. Therefore, we used mouse negative serum instead of human negative serum to calculate critical values. A total of 56 negative mouse serum samples were used in a single neutralization experiment to calculate the ED_{50} value. The cut-off values were defined as 30 and 183 for HCoV-229E and HCoV-NL63, respectively. Although research suggests the presence of sialic acid during the invasion of cells by HCoV-OC43 and HCoV-HKU1, the specific receptor has not been identified [1]. Pseudotyped OC43 and HKU1 had been reported by A.T. Sampson et al. [27], in which lentiviral pseudovirus were used. The production of pseudotyped OC43 and HKU1 was further modified by the addition of exogenous neuraminidase and co-transfection of TMPRSS2 in producer cells, respectively. Besides, VSV pseudotyped OC43 was also generated by C. Wang et al. [28]. And HRT-18 cells were used for titering. These studies indicate optimization of pseudotyped virus generation and/or titering cells were necessary for high titer pseudotyped virus. We have preliminary results showing that the HKU1 pseudotyped virus and OC43 pseudotyped virus could infect the LLC-MK2 cell line. Further validation and method optimization is required to establish the neutralization assay. We will renew our results if there is any further progression.

Hence, ELISA was utilized as an alternative to neutralization assays to detect antibody levels in serum samples for these two coronaviruses' S protein. Although the pathogenicity of these four coronaviruses is relatively low, they can cause severe clinical symptoms in individuals with compromised immune function. In any case, this study found that before and after the COVID-19 pandemic, the coronavirus infection rate was very high, the lowest antibody-positive rate was 33 % in 2010, and the lowest antibody-positive rate was 49 % in 2023. Moreover, limited research exists on the infection rates and antibody levels of these coronaviruses in the population. HCoV-NL63, HCoV-229E, HCoV-OC43 and HCoV-HKU1 have been circulating in the population for a considerable period. However, most studies have been conducted on individuals exhibiting clinical symptoms of the common cold, employing methods such as realtime fluorescence quantitative polymerase chain reaction (qPCR) and ELISA for detection. The positivity rates of OC43 and HKU1 S protein in the healthy population were found to be approximately 70.8 % and 25.0 %, respectively, according to relevant studies.

The differences in antibody levels between the two population groups may be attributed to either temporal or geographical factors. Additionally, there may be relevance to cross-reactivity; however, the current existence of cross-reactivity remains controversial [29,30]. Consider the fact that antibody targeting BA.5 / BF.7 strain has poor cross-reactivity with Wuhan strains and vaccines harbouring Omicron variants antigen was not available in China by the time blood samples were taken, so D614G S protein antibody positive individuals in our study were likely SARS-CoV2 vaccinated population while D614G antibody negative individuals were likely non-vaccinated population. In order to discuss whether SARS-CoV2 vaccines may cause cross-reactive antibodies to the other HCoV, we analyzed antibody positive rates to NL63, 229E, HKU1 in SARS-CoV2 vaccinated and non-vaccinated population. We found no difference in antibody positivity rates for these viruses, suggesting there is little cross-reactive antibodies to the other HCoV in SARS-CoV2 vaccinated individuals. Since antibody positive rate of OC43 S protein is 100 %, so OC43 was not analyzed.

However, this study still has several limitations. Firstly, we were unable to obtain serum samples from the same region at different periods. The differences in positivity rates among different regions may be attributed to regional variations and variations in sampling times. Secondly, due to the unsuccessful construction of pseudoviruses for HCoV-OC43 and HCoV-HKU1, we could only use ELISA methods for serum antibody detection, which may introduce certain differences compared to pseudovirus neutralization methods. However, some studies have shown that the results of the neutralization detection method are consistent with the ELISA method [31]. Finally, due to the high infection rates of HCoV-229E and HCoV-NL63, as well as the presence of maternal antibodies, obtaining negative serum samples was challenging, necessitating the use of animal serum to define the cut-off value. Therefore, the cut-off values established in this study are based on laboratory analytical sensitivity and do not necessarily reflect clinical diagnostic significance.

Antibodies targeting the S protein, including binding antibodies and neutralizing antibodies, are protective antibodies and are crucial for vaccine evaluation. Understanding the prevalence of pre-existing antibody levels and positivity rates in populations is essential for assessing vaccine efficacy. This is particularly crucial in the current development of coronavirus vaccines, where knowledge of the background positivity of protective antigen antibodies in the population is crucial. Furthermore, there is a lack of systematic research data on serum antibody levels within the population [32–39]. Our study provides positive rate data for these coronaviruses' S protein and emphasizes the importance of detecting relevant pathogens and promptly identifying potential highly pathogenic mutations. By assessing antibody levels against seven coronaviruses S protein in the population, especially for seasonal HCoV screening in two settlements in China, this study provides support for coronavirus-related research and vaccine development.

Ethics statement

All human serum samples were collected with informed consent for pathogen marker detection. The experiments on mice were conducted under the approval of the Institutional Animal Care and Use Committee at the National Institutes for Food and Drug Control (NIFDC). The experiments on mice were by the Guide for the Care and Use of Laboratory Animal [2023(B) 033, 2023(B)042].

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Qi Jiang: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Xi Wu:** Writing – review & editing, Validation, Investigation, Data curation. **Fangyu Dong:** Resources, Investigation. **Shan Qiao:** Methodology, Data curation. **Qiaoyun Shi:** Methodology, Data curation. **Changyong Jian:** Resources, Investigation. **Chen Chen:** Resources, Investigation. **Jiuyue Zhou:** Resources, Investigation. **Youchun Wang:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition. **Weijin Huang:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

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