Seroprevalence of a novel human canine coronavirus in Arkansas

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Abstract

Background: In 2010, a coronavirus surveillance study was conducted. Using partial sequencing, three canine/feline-like coronavirus were identified in nasal swabs of patients showing influenza-like symptoms in the State of Arkansas. Recently, Gray and collaborators reported a canine-like coronavirus infecting patients with pneumonia in Malaysia. Samples were fully sequenced, and the virus was isolated and adapted to cell culture. Based on the findings of Gray's group, serum samples collected in 2010 were revisited. These samples were from individuals living in the lower Mississippi Delta region in Arkansas, from ages 1 to 96 years (Median = 58), consisting of 57% females and 42% males.
Methodology: An ELISA antibody against the cell adapted CCoV-HuPn-2018 was developed to study cross reactivity to the virus discovered in Malaysia to determine if this virus is similar to the one detected in 2010 in Arkansas. Results: Preliminary results showed that serum samples collected in 2010 to have CCoV-HuPn-2018-specific IgG titers ranging from 1:256 to 1:4,096, with the highest average titers in serum from individuals under 20 years of age. Titers were also higher in males, compared to females. A virus neutralizing plaque assay and preliminary results showed that VN titers ranged from <1:64 to >1:1,024, the higher titers coinciding with the higher serum IgG titers.
Conclusion: This study will help to determine the seroprevalence of this virus to estimate if the samples detected in the nasal swabs in 2010 represented a small spillover of the virus or if this virus could have adapted to the human population in the State of Arkansas with more extensive circulation.

Introduction

To predict new emerging viruses, scientists have for many years sampled thousands of animals and still we failed in predicting the emergence of the COVID-19 pandemic. The emergence of a new virus in humans typically represents spillover from an animal virus. This process takes from years to decades to occur or may never occur. Spillovers are frequent; however, the outcome varies from infection of 1-2 persons followed by virus disappearance (dead-end infections) to widespread outbreaks. In a dead-end infection the pathogen causes disease in an individual, but further transmission to other hosts does not occur. Co-habitation or proximity of multiple host species can change the dynamics of dead-end infections, where if the infection is unsuccessful in one host species, the presence of other host species can provide alternative route for the pathogen to infect humans. With the earlier SARS-CoV infection, a bat coronavirus was transmitted to Asian Palm Civet and from there to humans, which fits the model of adaptation and spread from host-to-host.
A more recent concept, supported by Gray and Abdelgadir, is that scientists should focus on sampling spillovers, before virus adaption. The innumerous viruses isolated from animals may never spillover. Constant spillover of the same strains followed by virus evolution and adaption to a new host are required for the establishment of a new emergent virus.
Gray's group have recently reported an unknown coronavirus in human pneumonia cases in Malaysia. They described 8 patients harboring this coronavirus (CCoV-HuPn-2018) among 301 pneumonia patients. It appears that this virus could be considered the 8th coronavirus to infect humans. Another example is the report of a porcine deltacoronavirus in children in Haiti, a genus not yet reported in humans, where children attending a private school showed respiratory and diarrheal illnesses followed by acute undifferentiated febrile illnesses.
In 2010, NCTR scientists conducted an animal and human coronavirus surveillance study. A canine/feline like coronavirus was detected in nasal swabs from patients reporting influenza-like symptoms, but negative for influenza virus infection. It is possible that this virus is similar to the virus described by Gray's group and that this virus already circulates or is a spillover that occurred in Arkansas and may be similar to CCoV-HuPn-2018

Materials and Methods

Specimens. Ninety-five serum samples were obtained from the delta region in Arkansas from patients seeking diagnosis from a variety of diseases. All serum samples were heat-inactivated at 56ºC for 30min before analysis. Five plasma samples from convalescent COVID-19 patients were obtained from BEI resources.
Isotype-specific ELISA antibody titers. Plates were coated with semi purified and inactivated CCoV-HuPn-2018, HCoV-NL63, HCoV-OC43, HCoV-229E and HCoV-HKU1 or mock-infected cell culture supernatant in 0.1 M carbonate-bicarbonate buffer (pH 9.6) and incubated at 4°C overnight. The plates were blocked with 1% bovine serum albumin, and reagents and samples were added in the following order: (i) fourfold serial dilutions of serum, (ii) HRP conjugated antibodies to human IgG and tetramethyl benzidine (KLP). The reaction was stopped with stop solution (KPL) and OD at 605nm were measured with an EIA reader. Antibody titers were calculated as the reciprocal of the highest sample dilution which produced a mean absorbance greater than the cutoff value (the mean absorbance for the negative controls plus 3 standard deviations), after subtraction of the mean absorbance for the mock-coated wells from the of the antigen-coated well for each sample.
Plaque reduction Assay. Neutralizing antibodies were measured using a plaque reduction assay. The test was conducted in confluent cells grown in 6-well plates (A-72) and overlayed with agar. The neutralizing antibody titer was expressed as the reciprocal of the initial serum dilution that results in an 80% reduction in plaques.
Statistical analysis. The isotype-specific antibody and virus neutralizing antibody titers were compared by using the General Linear Model procedure with $\log_{10}$-transformed data. Statistical analyses were carried out using one way ANOVA, Tukey pairwise comparison and Parson's correlation (SPSS); significance was assessed at a *P* value of <0.05 throughout the study.

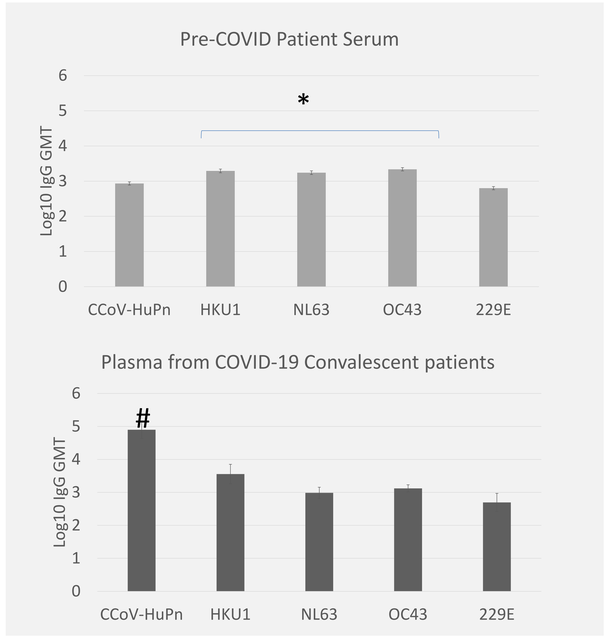

Figure 1. Coronavirus-specific IgG GMT of: A- pre-COVID serum from patients with flu-like symptoms and, B- Plasma from COVID-19 convalescent patients. * = Denotes HKU1, NL63 and OC43 GMTs significantly higher than CCoV-HuPn2018 and 229E (p<0.001) . #=Denotes CCoV-HuPn2018 log10 GMT significantly higher than the other four groups p=0.014

Results and Discussion

IgG specific antibody
All pre-COVID sera and COVID convalescent plasma had detectable levels of IgG antibodies to CCoV HuPn2018 and for all four common cold HCoVs (HKU1, NL63, OC43 and 229E).
IgG specific antibody to CCoV HuPn2018 was significantly higher in the plasma of convalescent COVID patients compared to antibodies in sera of pre-COVID patients. Among pre-COVID patients, sera from age group 0-19 , 20-49 and 50-69 years, antibodies to CCoVHuPn2018 and 229E were significantly lower than antibodies to all other common cold HCoVs (p<0.05). No difference was observed in the antibody levels in the age group of >70 years of age for the five tested viruses.
Virus Neutralizing antibodies
Sixty two percent of the pre-covid patient sera had VN titers to CCoV HuPn2018. There was no difference between gender or between age groups and VN titers.

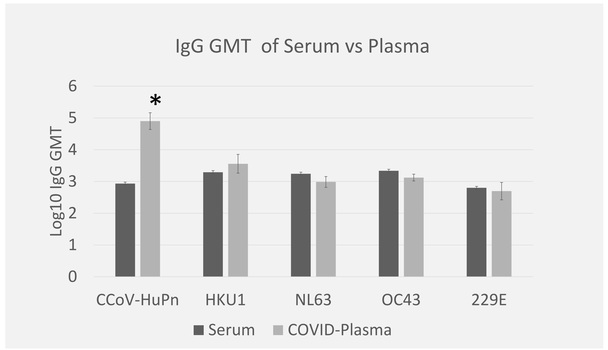

Figure 2. Comparison between coronavirus-specific IgG isotype in pre-COVID serum from patients with flu-like symptoms and Plasma from COVID-19 convalescent patients. * = Denotes CCoV-HuPn2018 GMTs significantly higher in plasmas of convalescent COVID-19 patients than pre-COVID patient sera (p<0.0001)

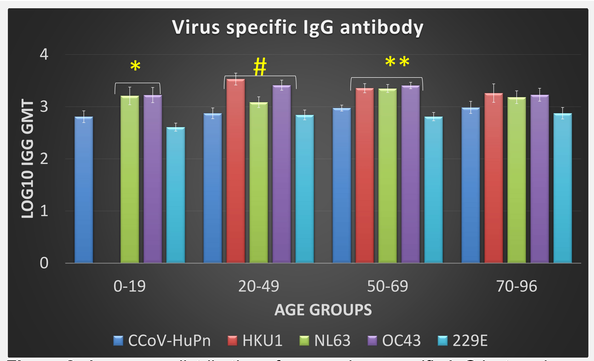

Figure 3. Age group distribution of coronavirus-specific IgG isotype in pre-COVID serum from patients with flu-like symptoms. *= Denotes HKU1 and NL63 significantly higher than the other groups (p <0.05). # and **= Denotes HKU1, NL63 and OC43 higher than the CCoVHuPn2018 and 229E (p=0.008 and p=0.003).

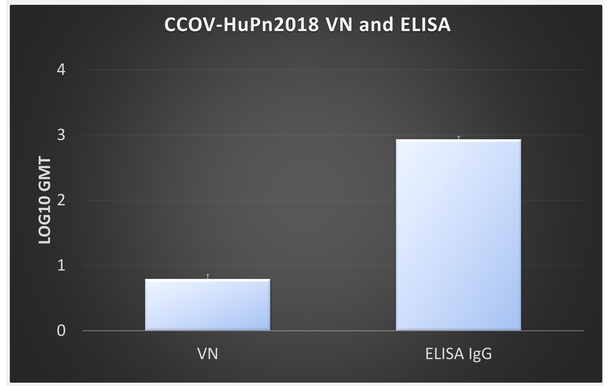

Figure 4. Comparison between CCoV-HuPn2018 specific IgG isotype and virus neutralization antibody in pre-COVID serum from patients with flu-like symptoms . There were no correlation between VN and ELISA IgG.

Conclusion

We detected both, IgG specific and virus neutralizing antibody against CCoV-HuPn2018 in the general population, demonstrating that CCoV-HuPn2018 or a closely-related virus circulated in Arkansas and infected humans in that period of time. Moreover, it appears that in COVID-19 convalescent plasma, antibodies to this virus may have been boosted by COVID infection, as observed by the significantly higher IgG titers detected in those samples compared to pre-COVID samples. To confirm these findings, we plan to extend the number of COVID-convalescent plasma or sera.
Our previous study of human nasal swabs from patients with flu-like symptoms showed that in the 2010/2011 flu season OC43 and NL63 were the most prevalent strains circulating that year. In this study, sera collected in the same period, showed higher IgG titers to OC43, NL63 and HKU1 corroborating the epidemiology of those virus in Arkansas.