Executive Summary: COVID-19
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Authors

Dr Doug Quarry
Professor John Oxford

The Executive Summary is produced weekly and contains in-depth analysis.
International SOS publishes a COVID-19 Daily Case Summary.

INTRODUCTION

In this week's edition of the International SOS COVID-19 Executive Summary we explore:

- Waning Countries
- Looks like there is only one strain of SARS-CoV-2
- Update on humoral and cell-mediated immunity
- Update on Texas
- Article / Journal roundup
- A View from the Lab: Professor John Oxford:
    - Speech droplets and transmission of COVID-19 transmission
    - The critical role of children in spread of COVID-19. Do they or do they not?
    - Personal reports of infection from medical scientists and virologists
    - Structure of the receptor binding domain and molecules of COVID-19 – x-ray crystallography studies
    - The future of long-haul travel?
    - Personal post-script

WANING COUNTRIES

As discussed last week, the map on the International SOS Pandemic site now contains a country classification of "Waning". Currently, waning countries are:

- Australia
- China
- Czech Republic
- Estonia
- Greece
- Iceland
- Luxembourg
- New Zealand
- Norway
- Slovenia
- South Korea
- Thailand

LOOKS LIKE THERE IS ONLY ONE STRAIN OF SARS-CoV-2.

Epidemiological implications

Applying molecular biology tools to traditional epidemiology has greatly improved outbreak monitoring and prevention for all types of viral diseases.

These tools include genotypic and phenotypic methods to determine the specific strain or type of virus circulating in a population. They can be used to improve diagnostics, to guide treatment programs and vaccine development, and to trace the spread of pathogens.

Some definitions

- **Nucleosides**: Nucleosides are nucleotides without a phosphate group.
- **Nucleotides**: a compound consisting of a nucleoside linked to a phosphate group. Nucleotides form the basic structural unit of nucleic acids such as DNA or RNA. A nucleotide consists of three things:
    - A nitrogenous base, which can be either adenine, guanine, cytosine, or thymine (in the case of RNA, thymine is replaced by uracil).
    - A five-carbon sugar, called deoxyribose because it is lacking an oxygen group on one of its carbons
    - One or more phosphate groups.
- **RNA**: a chain of nucleotides, usually single stranded folded onto itself (unlike DNA)
- **RNA virus**: a virus that has RNA (not DNA) as its genetic material. RNA viruses cause the common cold, influenza, SARS, COVID-19, hepatitis C, hepatitis E, West Nile fever, Ebola virus disease, rabies, polio and measles.
- **Genome:** the genetic material of an organism. It consists of DNA (or RNA in RNA viruses).
- **Gene:** Genes are small sections of DNA (or RNA in RNA viruses) within the genome that code for proteins
- **Sequencing RNA:** determining the order of the four nucleotides
- **Codons:** A unit of three nucleotides that code for a single amino acid.
- **Amino acids:** the basic building blocks of proteins
- **Antigen:** a harmful substance which enters the body which causes the body to make antibodies as a response to fight off disease
- **Protein:** a protein is a substance that has amino acids and other compounds (carbon, hydrogen, oxygen, nitrogen and sometimes sulfur)
- **Mutation:** a change in the viral genetic code (RNA code sequence). Most mutations are deleterious and only some beneficial to the virus
- **Mutation rate:** the probability that a change in genetic information is passed to the next generation.
- **Strain:** a genetic variant of an organism that produces at least one different protein. For example, in influenza, mutations can change the surface glycoproteins: hemagglutinin (H) and neuraminidase (N).
- **Pathogenicity: the ability of an organism to cause disease**
- **Phenotype:** the set of observable characteristics resulting from the interaction of a genotype with the environment
- **Phylogenesis:** the evolutionary development and diversification of a feature of an organism.
- **Viral phylodynamics:** the study of how epidemiological, immunological, and evolutionary processes act and potentially interact to shape viral phylogenies

Mechanisms of viral mutation and sequencing

- Viral replication is error-prone and causes SARS-CoV-2 (and all other viruses) to mutate (undergo small changes in the genome)
- The vast majority of mutations “break” the virus so that it cannot transmit and/or replicate, or make no change to its function (redundant code)
- Rarely a change in the code helps a virus to replicate and/or transmit better – but the majority of these changes have only a tiny effect. Despite this, RNA viruses rapidly accumulate genetic variation because of short generation times and high mutation rates
- Patterns of viral genetic variation are also affected by selection acting on viral phenotypes
- Traditional phylodynamic studies of viruses tended to focus on a limited number of viral phenotypes, commonly changes in transmissibility, pathogenicity and changes to antigens
- Rapid and repeated analysis of the viral genome now allows mutations and related phenotypes to be tracked through an epidemic / pandemic both temporally and geographically.

Accurate sequencing

Accurate sequencing is key to producing high-quality genomes for analysis. Dramatic improvements in high-throughput (also known as next-generation) sequencing technologies and in virus-specific sequencing in the past decade have enabled sequencing of viruses, known and novel, from all kinds of samples.

There is only one strain of SARS-CoV-2

The differences in the genomes between viruses can be used as “breadcrumbs” to trace the history of a given sample as it will contain unique combinations of genetic mutations.

A recent report from nextstrain.org has concluded:

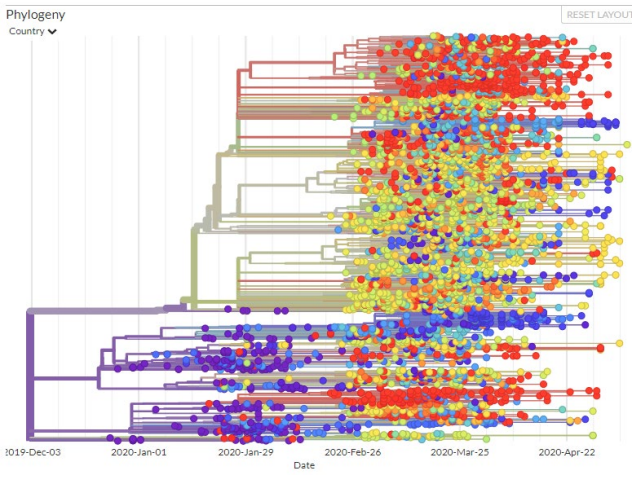
1. The evolutionary rate of SARS-CoV-2 is typical for a coronavirus

2. There is only one strain of SARS-CoV-2
   - Note that this is a sequencing-based conclusion and phenotypic changes were outside the parameters of the study

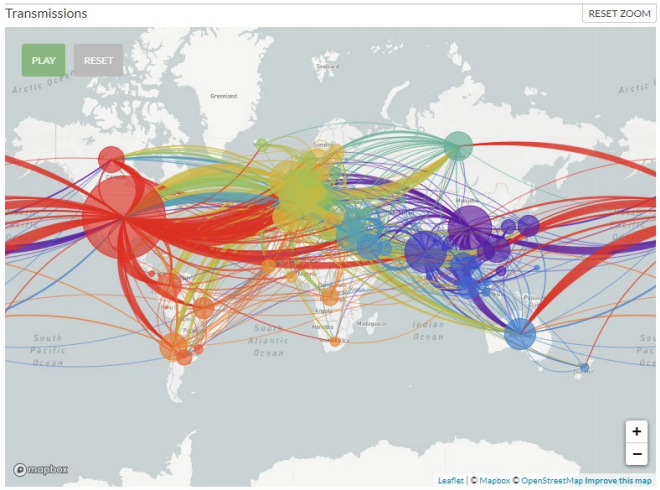
3. D614G may be related to transmissibility, but could also be explained by geography
   - However, this could also just be a side effect of the natural history of the pandemic. See detailed explanation.

Nextstrain.org: Genomic epidemiology of novel coronavirus – Global subsampling

Phylogeny (worth watching)



Transmissions (worth watching)



Analysis

The differing epidemiology of COVID-19 around the world (cases, clusters, outbreaks, crises, etc.) apparently cannot be explained by viral differences; other explanations must be found.

- Do crises (NYC / Lombardy / UK) require weeks of unrestrained viral transmission to build up?
- Do the relatively "lightweight" but successful interventions in Japan mean that the R_0 can be brought to less than one without lockdown?
    - It has been suggested that this may be because Japan it is a combination of a highly disciplined and a society-focused nation assisted by a primary healthcare network set up to combat TB in the early 1950's (which also means everyone has had BCG vaccination)
- Does the lack of a significant outbreak in Jilin (China) mean that strict early interventions work?
    - It has been suggested that Jilin demonstrates that the single most important step to take for governments easing restrictions is to increase testing, enabling an early clamp down on potential resurgence of the virus

- We look forward to better understanding the disease and its epidemiology in coming weeks.
- We also look forward to further analysis of this quality.

UPDATE ON HUMORAL AND CELL-MEDIATED IMMUNITY

A new paper in the journal "Cell" describes T cell and antibody immune responses in average COVID-19 cases.

The study found:

- 100% of COVID-19 cases made antibodies
- 100% of COVID-19 cases made CD4 T cells
    - These help coordinate the immune response by stimulating other immune cells, such as macrophages, B lymphocytes (B cells), and CD8 T lymphocytes (CD8 cells), to fight infection.
- 70% of COVID-19 cases made measurable CD8 T cells
    - These play an important role in eradicating virus-infected cells and tumor cells

These findings are interpreted as good news and consistent with normal viral immunity

The CD4 T cell responses to the spike protein, the main target of vaccine efforts, were robust and correlated with the level of the anti-SARS-CoV-2 IgG and Ig A titers. Again, interpreted as good news.

The study chose people who had had an "average" COVID-19 course – non-hospitalized – to provide a benchmark of what a normal immune response to SARS-CoV-2 looks like.

Cross-reactivity

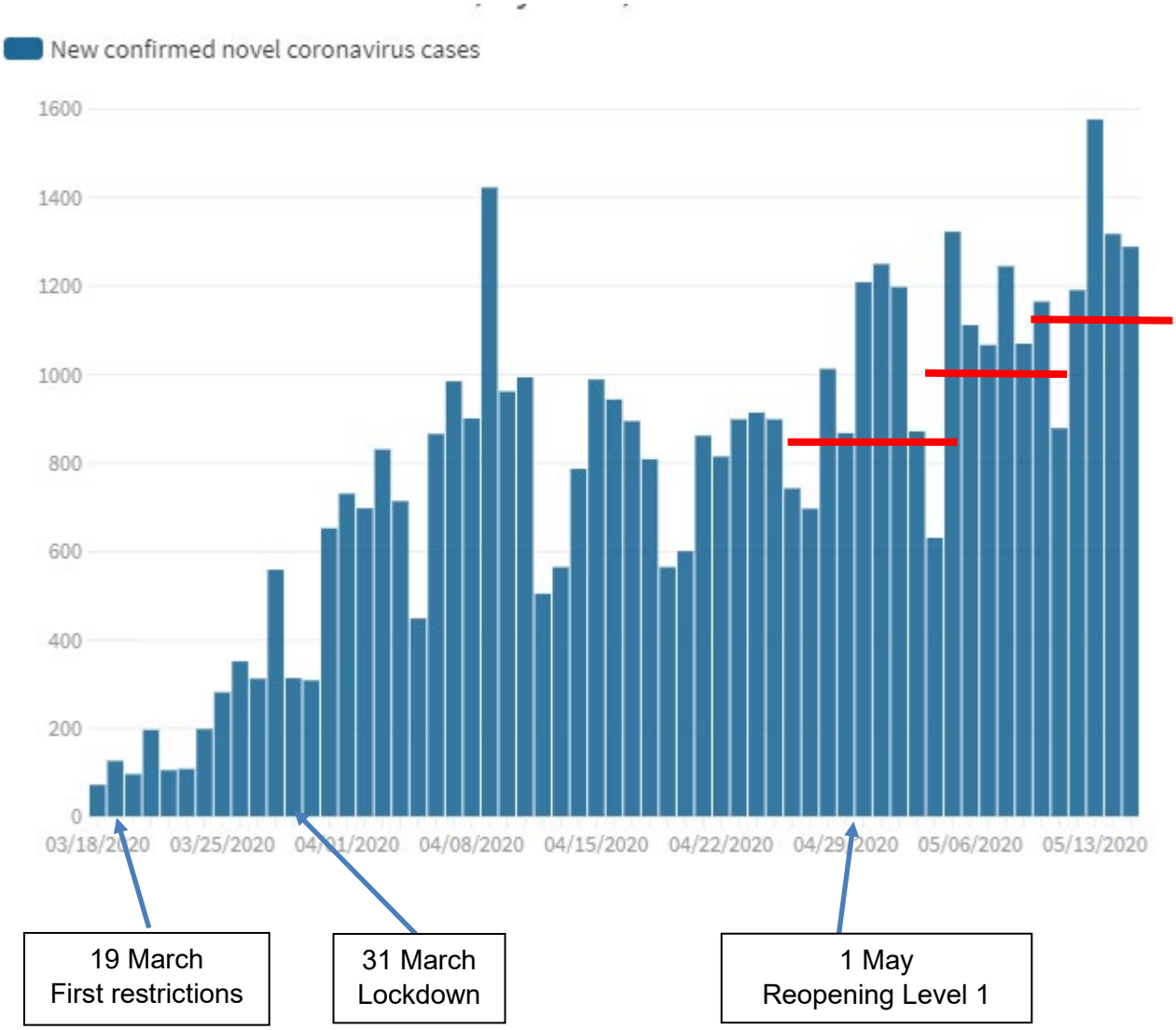
Very importantly, the study detected SARS-CoV-2-reactive T cells in approximately 50% of unexposed people suggesting cross-reactive T cell recognition between circulating "common cold" viruses and SARS-CoV-2. This may influence an individual's susceptibility to COVID-19

Cross-reactive T cells are also important to vaccine development as cross-reactive immunity could influence responsiveness of candidate vaccines.

UPDATE ON TEXAS

This is our third weekly report on the number of cases per day in Texas since the “re-opening” in stages on 1 May. The graph below from the Houston Chronicle shows an apparent increase of daily confirmed cases to about 1,500 per day. We will continue to report on Texas as a possible microcosm of the USA.

Texas: new confirmed cases: Houston Chronicle



ARTICLE / JOURNAL ROUNDUP

WHO advises against spraying or fumigation

The WHO has advised against spraying disinfectants outside or across broad spaces indoors to try to kill coronavirus, saying it can do more harm than good.

WHO commented that:

- Spraying or fogging of certain chemicals, such as formaldehyde, chlorine-based agents or quaternary ammonium compounds, is not recommended due to adverse health effects on workers in facilities where these methods have been utilized
- When used outdoors, disinfectant is inactivated by dirt and debris and it is not feasible to manually clean and remove all organic matter from such spaces
- Spraying disinfectants can result in risks to the eyes, respiratory or skin irritation and the resulting health effects

Image: Reuters

Moderna's vaccine promising

Moderna's coronavirus vaccine is one of eight vaccines that have started tests on humans.

The 45 volunteers were divided into three groups, receiving different doses of the vaccine. All were given an initial dose and a booster one month later. Preliminary data (not published) shows that all 45 volunteers developed antibodies, eight participants (all who were tested) and who had received low and medium doses of vaccine developed neutralizing antibodies. Phase II will involve 600 volunteers, half older than 55.

Moderna, Inc. developed the vaccine in collaboration with the National Institute of Allergy and Infectious Diseases, of which Anthony Fauci MD is Director.

30 million doses of vaccine for UK by September?

A deal between Oxford University and AstraZeneca could produce 30 million doses of a COVID-19 vaccine by September. The British Government has pledged £65.5 million additional funding for the project.

What is happening on the Theodore Roosevelt?

The USS Theodore Roosevelt has been docked in Guam for nearly two months after more than 1,200 sailors were infected in a coronavirus outbreak in March 2020.

Presently nearly 3,000 sailors are returning to the ship. Officials have said that at least 14 returnees have tested positive for the virus. An undisclosed number of sailors testing positive had been infected in the first outbreak, had recovered, and had tested negative prior to their return to the ship.

It is reported that Defense officials are not sure whether some of the tests had produced false negative readings and/or whether some of the sailors had contracted the virus after spending two weeks in quarantine in Guam. We await further reports.

Image: New York Times

Dogs sniffing out COVID-19?

A trial in the UK to determine if medical sniffer dogs can detect coronavirus in humans will start soon. The trial to be led by the London School of Hygiene & Tropical Medicine and Durham University, has received £500,000 of government funding. If successful, dogs could become part of the government's wider testing strategy.

Rice ATMs in Indonesia

The Indonesian Government is rolling out so-called "Rice ATMs" across Jakarta to help the needy during the COVID-19 crisis. Ten machines have been installed so far – each dispenses 1.5kg of rice to the poor. The machines can dispense up to 1.5 tonnes of rice per day for 1,000 hungry residents. Indonesia has operated a large-scale rice subsidy scheme since the 1990s Asian financial crisis known as "Raskin", or "Rice for the Poor."

Image: ABC

The Oxford vaccine provided only "non-sterilizing" immunity in monkeys

Soon after data appeared from Sinovac, the maker of an inactivated virus vaccine, reported protection of rhesus monkeys, the Jenner Institute in London, maker of an adenovirus-based vaccine with a SARS-CoV-2 spike protein, issued a press release also showing protection of rhesus monkeys and said they were moving forward to large-scale human trials.

Actually, all vaccinated monkeys in the Jenner trial **did** become re-infected when challenged, and RNA was obtained from nasal secretions. In addition, the level of neutralising antibody was low.

However, it appears that the vaccine did moderate the severity of their disease, as measured by respiratory rate and degree of lung damage. This type of protection is called "non-sterile" immunity, and at some point, it may be that partial protection will be judged sufficient to control the COVID-19 pandemic. Watch this space.

A VIEW FROM THE LABORATORY: PROFESSOR JOHN OXFORD

Speech droplets and transmission of COVID-19 transmission

In our reports recently we placed some emphasis on transmission of the new virus from asymptomatic patients by simple tidal breathing. Many studies have confirmed droplet and even aerosol transmission from coughs and sneezes, but the possibility of virus transmission from ordinary breathing has been largely ignored. In the last years two papers have investigated this opportunity for virus spread and my own quarantine group in London helped with one of the studies looking at prevention and climatic influences. We were part of the EMIT Consortium.

Now a new study (Stadaytckyi *et al*, The airborne lifetime of small speech droplets and the potential importance in SARS-CoV-2 transmission, NIH University of Pennsylvania, PNAS, May 13th) used laser scattering to show that "loud speech can emit thousands of oral fluid droplets per second. The authors conclude that in the closed stagnant air environment they survive for 8-14 mins and the droplets are 4μm in diameter. The study indirectly confirms the old family doctor's advice of the past which was to open the windows of the sickroom! '

Droplet nuclei have a diameter of less than 30μm, and once airborne, they dehydrate quickly by evaporation. The experimenters detected 2,600 small droplet nuclei emitted per spread when speaking. On average viral load of $7x16^6$ per ml one minute of loud speaking would generate 1,000 virus-containing droplet nuclei which would remain airborne for at least 8 minutes and therefore potentially be inhaled. The authors felt that their values were a conservative lower limit estimate'. Note that these droplet nuclei can reach the lower respiratory tract.

These authors working at NIH and the University of Pennsylvania used an intense “sheet of laser light” to visualize and film bursts of speech droplets. Peak emission rates were 10,000 s^{-1}. Medium sized droplets (10-100µm), remained airborne for 30 secs. They noted that once airborne, speech-generated droplets rapidly dehydrated because of evaporation and decreased in size which slowed their fall.

The oral fluids include electrolytes, DNA, enzymes, and remnants of dehydrated epithelial cells. On a stagnant-air day, they predicted the droplet nuclei from speaking persisted as a slowly descending cloud emanating from the speaker’s mouth.

They had a 226 titre enclosure and a fan spread the particles. There was a temperature gradient of 0.5^{o}C because of heat dissipated from the iPhone II camera, which was attached to the front of the enclosure. The enclosure box was firstly purged of with HEPA filtered air and then the enclosure filled with speech droplets with a repeat of the phase “stay healthy” for 25 sec. A movie clip was analysed from frame by frame to quantify the number of spots and streaks. The number of drops was 2,600 small droplet nuclei per second of speaking. They estimated that 1 minute of loud speaking generates at least 1,000 virion containing droplet nucleic that remain airborne for more than 8 minutes. The possibility is that such a droplet could be inhaled and trigger a new COVID-19 infection. The authors consider that they have shown lower limit estimates. With a higher virus titre in saliva, the emitted droplets could reach over 100,000 per minute of speaking.

There is an unexpected sequel of this paper published in the Observer on 17 May by Robin McKie, the Science Editor, who describes how a choir in Amsterdam gave one of the last performances there before lockdown. In the days that followed, 102 of 130 choristers became ill with COVID-19, one person died, and three partners of the choir were admitted to intensive care. Other choirs had similar high attack rates. A study then carried out in Munich of choir singing and also emissions from musical instruments showed that the flute, oboe, and clarinet posed the most serious infection problems.

Of course, it is possible that in each case, the virus was spread at social events before and after the concert. But post-COVID-19, I feel choral and wind instrument concerts will be viewed with some caution!

The critical role of children in spread of COVID-19. Do they or do they not?

At present many countries including the UK are planning to allow children back to school after a shutdown. Many politicians tend to hide behind “science”, but here the “science” is rather conflicting. Last week we quoted Christian Drosten’s data from Berlin, which found that children may be as infectious as adults. In Australia there is some data that transmission from school children is limited and lower than for influenza (reviewed by Mallapaty, Nature 581, 127).

Everyone agrees that children have mild symptoms with COVID-19, although there are concerns that a low percentage of infected children can exhibit an unusual inflammatory response similar to Kawasaki Disease. Immunologists are now studying the details of the immune response for COVID-19 in children and there is preliminary data to suggest that sicker children, and indeed adults, do have higher cytokine levels.

Personal reports of infection from medical scientists and virologists

Unusually, in my experience, obituaries are mentioning COVID-19 in their text. Also, virologists are describing their personal experiences. This was started by N. Ferguson, a mathematical modeller in London who had broken the government's guidelines of social distancing and had to resign.

Prof Peter Piot, Co-discover of Ebola Virus and Director of the London School of Hygiene and Tropical Diseases described this week his own clinical experience with COVID-19, and today Paul Garner, from the Liverpool School of Tropical Medicine, enlarged on his own protracted infection.

At the same time Tim Spector, of King's College, has reported that his group has a tracker system which has picked up 200,000 patients where symptoms have persisted for six weeks. These are community-acquired cases. The app has tracked 15 different symptoms and a distinct pattern of waxing and waning has been noted "COVID is the strangest disease of my career". He noted scientifically an explanation that it is a multi-system disease potentially infecting many organs. Prof Garner at Liverpool has experienced 'different symptoms everyday', breathlessness, dizziness, arthritis of the hands. "This is a novel disease with strange pathways and an outrageous one".

Perhaps as a very English post-script; labradors and cocker spaniels are being trained to detect the smell of COVID-19 infection under a government grant at Durham University and the London School of Hygiene and Tropical Diseases. The objective is for 250 persons to be screened per hour. Certainly, in the Spanish influenza in 1918, clinicians described the very unique odour in the influenza ward and the patients themselves.

Structure of the receptor binding domain and molecules of COVID-19 – x-ray crystallography studies

Two side-by-side papers in Nature (Lan *et al* p125 and Shang *et al* p229) describe the crystal structure of the spike protein of COVID-19 virus bound to the cell receptor, ACE-2. These are extremely detailed papers but are worth our perseverance. After all, by examining the receptor-binding domain (RBD) of SARS, COVID and other B-Coronaviruses we will be able to establish evolution of the new viruses and origin, and also prepare cross reactive antibodies and vaccines and antivirals to react with the virus RBD. Already it is clear that a bat coronavirus called R_0 TG13 shares 93% of the spike S gene. SARS is more separate, sharing only 80% sequence identity.

The group investigated the interaction between COVID-19 RBD and ACE-2 at high resolution using x-ray crystallography. The COVID-19 RBD has a twisted five stranded anti parallel B sheet with short connecting helices and loops that form the core. One notable common feature that was found for both RBD-ACE interfaces is the network of hydrophilic interactions. There are 12 hydrogen bonds and 2 salt bridges at the interface. The other feature is the involvement of multiple tyrosine residues that form hydrogen bonding interactions with the polar hydroxyl group. The third shared feature is in the Asn90 linked glycans of the ACE-2 that bind to different RBDs.

In the COVID-19 RBD-ACE-2 structure the first four N-acetyl-β-glucosaminide (NAG) glycans were linked to ACE-2 Asn90. Glycans after the first NAG may interact with COVID-19 RBD and have an important role in binding. Shang *et al* using similar technology have identified two virus binding hot spots at the RBD-ACE-2 interface. They showed that a bat coronavirus Ro TG13 also uses ACE-2 as a receptor.

COVID-19 has structural changes in the ACE-2 binding ridge caused by a 4 residue motif, namely residues 48-485 and this allows the ridge to become more compact and to form better contacts with the N terminal helix of ACE-2. Neutralising the changes of lysine is key to the binding of coronavirus RBD's to ACE-2.

The future of long-haul travel?

A Hong Kong journalist Laurel Chor (Observer newspaper) took a 12-hour flight from Hong Kong and this was extended by 7 hours for medical checks. Could this be one awesome look at the future of air travel? The BA staff at Heathrow had masks and gloves. All the passengers wore a mask, and some had full body protective suites and plastic face shields, goggles, ,and gloves. In Hong Kong, the passengers were transferred from station to station, downloading apps and being provided with tracking bracelets. Phone numbers were checked, and a thermometer provided for twice daily temperatures. They were then moved to a Convention Centre converted for testing with video instructions. Ms Chor spat into a vial and was led to a final hall and was relieved as the sample was negative. Finally, she went home to be quarantined for 2 weeks.

Personal post-script

For two years I have been recording stories from 1918 for the radio programme "Made in Manchester" and these recordings are being transmitted on BBC Radio 4. The three episodes are about the 1918 influenza where of course nearly 100 million people died. But 95% of the population survived as the virus attacked the younger age group and left the older segment alone.

AUTHORS

Dr Quarry MBBS, MSc (Community Health):
Group Medical Director, Health Intelligence, International SOS

Professor John Oxford PhD, DSc, FRCPE
International Virologist & Author
Blizzard Institute, Queen Mary College, London.
Scientific Director, Oxford Media Medicine