COVID - 19

CORONA VIRUS: WHAT DO WE KNOW?

***Jaydeep Choudhury**
****Dhanalakshmi K**

Abstract: *Coronavirus causes a wide variety of diseases in various animal species. It is known to cause innocuous respiratory infections and occasional viral diarrhea in humans. Pandemic caused by SARS-CoV-2 (a beta corona virus) is a third spill over in two decades of an animal corona virus to humans. It uses ACE2 receptors for cell entry. Active viral replication has been proved in the cells of human respiratory tract, conjunctiva and gastrointestinal tract contributing to multiple routes of transmission. Peak viral load is noted at the time of presentation which explains the transmission even in pre-symptomatic stage. R_O is expected to be around 2 to 3, which explains the higher pandemic potential. The virus persists on inanimate objects for a variable period of time depending on the infectious dose, temperature and humidity.*

Keywords: *Coronavirus, Basic reproductive number, Viral load, Replication sites, Infectivity, Stability.*

The Coronavirus family comprises of two subfamilies, Coronavirus and Torovirus. The Coronavirus subfamily is divided into four genera, alpha, beta, gamma and delta. Human Coronaviruses (HCoV) belong to alpha and beta genera. Following corona viruses are found to have the pandemic potential i) SARS-CoV-1, ii) MERS and iii) SARS-Cov-2, this is the virus responsible for the current pandemic. The first HCoV isolation was reported in 1965.The first epidemic of HCoV, Severe Acute Respiratory Syndrome (SARS) was reported in 2002. Middle East Respiratory Syndrome (MERS) was the next major HCoV outbreak which occurred in 2012. In various animal species, a wide variety of diseases for causes. In chicken, it causes bronchitis and nephrosis. Manifestations in pigs include gastroenteritis and encephalitis. In dogs, turkeys and calves, enteritis is the usual presentation. While hepatitis and encephalitis in rats, peritonitis in cats, pneumonia and hepatitis in whales of the deseases in defferent specious. It has got varied manifestations in bats with multiple strains.[1] Recent available literature is reviewed for better understanding of the nature of virus and viral dynamics which will be useful for improving clinical care and containment of the disease.

* Professor, Department of Pediatrics, Institute of Child Health, Kolkata.

** Junior consultant, Department of Pediatric Infectious Diseases, Kanchi Kamakoti CHILDS Trust Hospital, Chennai.

email: drjaydeep_choudhury@yahoo.co.in

Human corona viruses

Coronaviruses are medium to large enveloped RNA viruses. It has a characteristic widely spaced, petal shaped surface projections, making the virus look like solar corona. The viruses are heat labile and also vulnerable to lipid solvents and alkaline pH.

Coronavirus is positive sense, single stranded RNA of 30 kilobases in length. (Single stranded RNA viruses are classified as positive or negative depending on the sense or polarity of the RNA. The positive-sense viral RNA genome can serve as messenger RNA and can be translated into protein in the host cell). It is the largest known viral RNA.[2] Structurally a nucleoprotein (N)

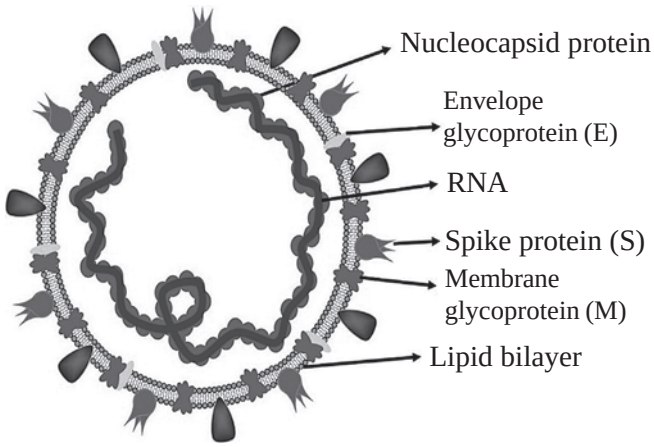

Fig.1. Human Coronavirus - Structure

Source: Shereen M A, Khana S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. J Adv Res 2020; 24:91-98.

surrounds the RNA genome and together they appear as a coiled tubular helix inside the bilayer lipid envelope, which anchor membrane (M), envelope (E) and spike (S) protein. A subset of corona viruses (specifically the members of beta corona virus) have a shorter spike-like surface protein called hemagglutinin esterase (HE).

While replicating, the virus attaches to the cell membrane by HE or S protein in the spikes. Some of the viruses use angiotensin-converting enzyme 2 (ACE 2) as the cellular receptor. Next the penetration occurs due to fusion of the viral envelope with plasma membrane. A large polyprotein is formed, cleaved into 15 or 16 nonstructural proteins and a replication complex is formed, following which the transcription is initiated. Virions are assembled by budding into cytoplasmic vesicles and released by cell lysis.

Epidemiology

Coronavirus infection may occur throughout the year, more cases are seen around winter months. It contributes to about 35% of upper respiratory infection during peak activity. Occasionally there may be outbreaks of infections. Reinfection is common which may be due to rapid diminution of antibody level after infection.[2]

Age

Among patients with common cold across all age groups, 2-10% are due to human corona viruses. Asymptomatic and symptomatic infections occur at all ages.

Transmission of HCoV

Infections occur through respiratory route. Aerosols are generated during cough, sneeze or even while talking. It consists of saliva and nasopharyngeal secretions that are contaminated with infectious agents. The droplets can be propelled for some distance depending upon their size and force of expulsion. The expelled droplets can land directly on the conjunctiva, oro-respiratory passage or skin of a close contact.

Small droplets less than 5μm can travel rapidly and to some distance depending upon the external environment. During dry season with less humidity, the moisture in these particles evaporate to produce droplet nuclei which are light and can remain airborne for a long time. Respiratory droplets can also contaminate inanimate objects. Touching these objects with contaminated fingers following cough or sneeze can transmit infection.

In healthy children, HCoV replicates only in the upper respiratory tract. The incubation period is generally 2 days and the infection lasts for about a week. Infection in immune-compromised children may be severe.

Manifestations of HCoV

(a) Upper respiratory tract infection: HCoV often presents like an undifferentiated acute respiratory tract infection. Rhinorrhea, sore throat, cough, malaise, headache and fever are the usual features.

(b) Lower respiratory tract infection: HCoV is the third most common etiology of viral pneumonia and bronchiolitis after respiratory syncytial virus (RSV) and parainfluenza virus. It may also precipitate acute asthma. HCoV may affect neonates and clinically present with apnea, hypoxia and bradycardia.

(c) Enteric infection: There are reports of nursery outbreaks of severe diarrhea and necrotizing enterocolitis (NEC) related to HCoV.[1]

(d) Neurologic diseases: HCoV is linked to neurological diseases like acute disseminated encephalomyelitis (ADEM), multiple sclerosis and polyradiculitis.

Severe acute respiratory syndrome (SARS)

SARS CoV-1 was first identified in China in November 2002 and subsequently it spread throughout the world. The epidemic lasted till the summer of 2003, the last known case occurred in summer of 2004. It accounted for 774 deaths (9.6% mortality) all over the world.[3]

SARS-CoV was classified as beta coronavirus lineage B. It originated in animals, most probably bats and then spread to exotic animals which were consumed by human in China. Humans were affected subsequently through an intermediate host, probably palm civet or raccoon dog. The viruses have been noted to mutate frequently and infect new species. SARS-CoV virus was transmitted by aerosols. It uses angiotensin-converting enzyme as a cellular receptor.[2]

In children, the disease manifested with fever, cough and systemic influenza like symptoms. Some children had diarrhea also.[1] Pneumonia developed in few children, mostly adolescents. Chest radiology showed ground glass opacities with peripheral consolidation. Maternal SARS-CoV infection resulted in maternal and fetal morbidity and mortality.

Lymphopenia with normal or decreased neutrophil count is the usual finding in peripheral blood examination. Neutrophilia is associated with poor outcome.[2] CPK, LDH and SGOT are usually abnormal. Reverse transcription polymerase chain reaction (RT-PCR) specific for

SARS-CoV in respiratory secretions is the confirmatory investigation. There is no specific treatment. Prevention is the mainstay. The epidemic was controlled by massive efforts at case identification and containment.

Middle east respiratory syndrome (MERS)

The first case of MERS was in Saudi Arabia reported in June 2012. Later it spread to different parts of the world. The virus was named MERS-CoV. Globally it accounted for 609 deaths (36% mortality).[4]

MERS-CoV was classified as beta coronavirus lineage C and is closely related to bat coronaviruses. All MERS-CoV infections were traced to middle east countries, mainly Saudi Arabia.[4,5] Initial transmission pattern of the virus showed reproductive coefficient (R_0) less than 1, which indicates low pandemic potential. Later, in one outbreak, superspreading was observed where one patient infected 80 individuals.[2] Mean incubation period was 5 days with a range of 2 to 14 days.

Patients suffering from MERS present with fever, chills, sore throat, cough, arthralgia and myalgia. They often develop dyspnea and rapidly progress to pneumonia. Many patients required ventilator support. Some presented with nausea, vomiting and diarrhea. Renal failure,

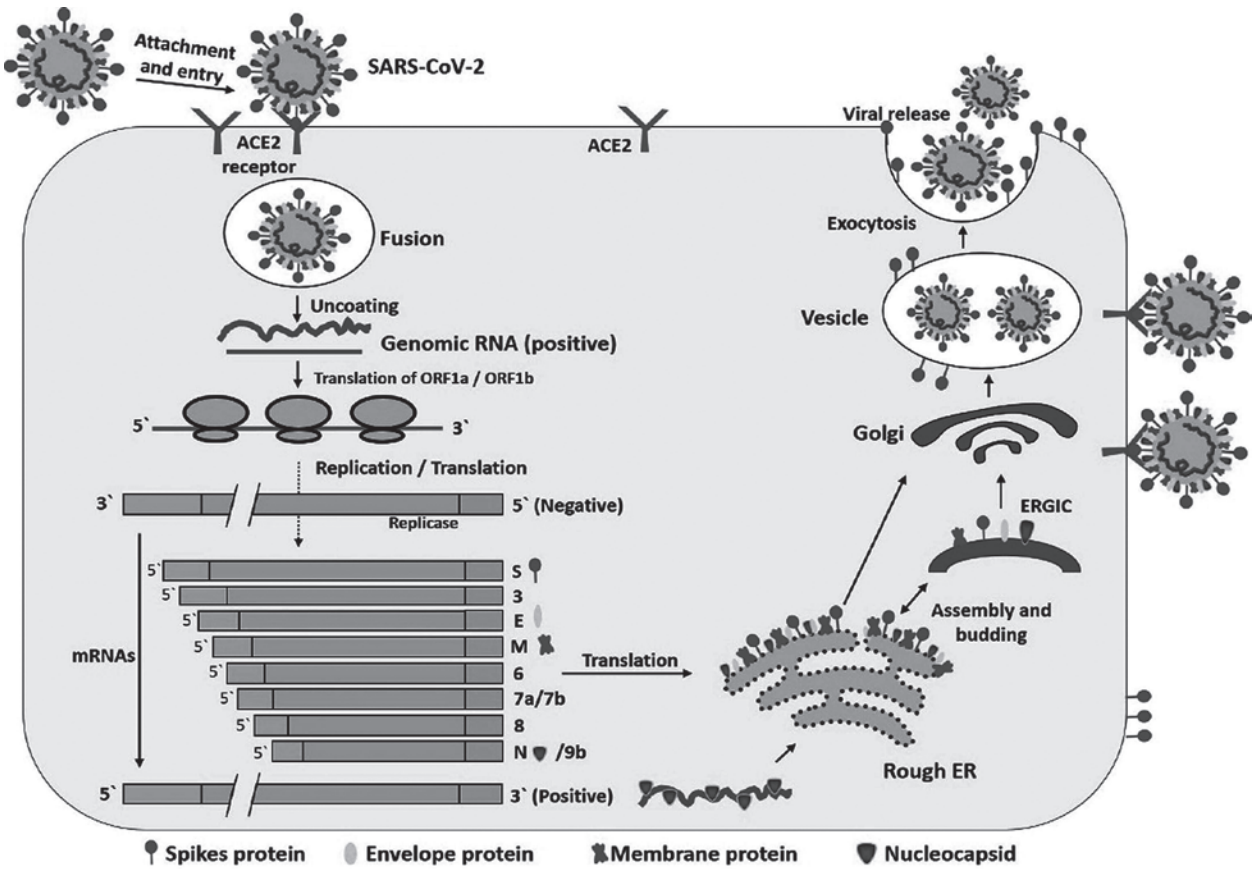

Fig 2. Life cycle of SARS CoV2 in host cells (begins its life cycle when S protein binds to the cellular receptor ACE2. After receptor binding, the conformation change in the S protein facilitates viral envelope fusion with the cell membrane through the endosomal pathway. Then SARS-CoV-2 releases RNA into the host cell. Genome RNA is translated into viral replicase polyproteins pp1a and 1ab, which are then cleaved into small products by viral proteinases. The polymerase produces a series of sub genomic mRNAs by discontinuous transcription and finally translated into relevant viral proteins. Viral proteins and genome RNA are subsequently assembled into virions in the ER and Golgi and then transported via vesicles and released out of the cell. ACE2, angiotensin-converting enzyme 2; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment).

Source: Shereen MA, Khana S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission and characteristics of humancoronaviruses. J Adv Res 2020; 24:91-98.

pericarditis and ARDS have been reported. Children and adolescents with MERS, occasionally, have been asymptomatic or mildly symptomatic.[1]

Both SARS and MERS have presented with similar clinical features. But patients with MERS have a shorter time from onset of illness to clinical presentation, enhanced requirement for ventilatory support and higher case fatality rate.[4]

SARS-CoV-2

The current pandemic caused by SARS -CoV-2 which emerged initially in Wuhan, China is rapidly spreading and so far has affected 216 countries with 3,00,441 deaths (as on May 16, 2020).[6] It was initially named as 2019 novel coronavirus because of the incomplete match between the genomes of this and other (previously known) coronaviruses.[7] This pathogen was later renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group and the disease was named coronavirus disease 2019 (COVID-19) by the WHO.[8] The incubation period of SARS-CoV-2 is estimated to be between 1 and 14 days, with a median of 5 to 7 days.[9]

Phylogenetic analysis of the SARS-CoV-2 genome indicates that the virus is closely related (with 88% identity) to two bat-derived SARS-like coronaviruses collected in 2018 in eastern China (bat-SL-CoVZC45 and bat-SL-CoVZXC21) and genetically distinct from SARS-CoV (with about 79% similarity) and MERS-CoV.[7] Even though findings suggest that bats might be the original host of this virus, further studies are needed to elucidate whether any intermediate hosts have facilitated the transmission of the virus to humans.[7,10]

Viral dynamics of SARS-CoV-2

Viral load from respiratory samples

In an analysis of a cohort of 23 patients with confirmed COVID-19 infection, peak viral load was highest at the presentation (5.2 $\log_{10}$ copies per ml) even when the disease is mild, explaining the high contagiousness of the disease.[11] Viral load gradually declined over second week. The relationship between the viral load, severity of the disease and mortality is yet to be ascertained. The reported median viral load of 1 $\log_{10}$ was higher in severe cases than mild cases and the difference was not significant.[11]

In another study from Germany of nine virologically confirmed cases, pharyngeal viral shedding was very high during the first week of symptoms (peak at day 4) and gradually declined (Box 1). The swabs taken after day 5 had a detection rate of only 39.93% [12]

Box 1. Viral RNA load from upper respiratory samples[12]

Peak on day 4	: 7.11×10^8 copies/swab
Average till day 5	: 6.76×10^5 copies/swab
Average after day 5	: 3.4×10^5 copies/swab
Viral load from lower respiratory samples (sputum)	
Maximum viral load	: 2.35×10^9 copies/ml
Average	: 7.00×10^6 copies/ml

Infectivity and replication sites

Presence of viral subgenomic mRNA indicates actively infected cells since subgenomic mRNA is transcribed only in infected cells.[12] When samples from the upper respiratory tract were analyzed the presence of high viral load proved that active viral replication is happening. Active viral replication was noted till day 5 of symptom onset and no subgenomic mRNA was identified after that.[12]

Similarly, active viral replication from lower respiratory samples (sputum) was obvious from Day 4 to Day 9 (which was evident from the levels of viral subgenomic mRNA). Decline in viral load occurred from Day 10 to Day 11. When analyzing the genotypes from throat and sputum, the presence of genotype distinct serotypes support the fact that viral replication is happening in the throat rather than shedding of the virus to throat from lung.[12] A recent study showed SARS-CoV-2 infected the ciliated, mucus-secreting, club cells of bronchial epithelium and alveolar cells in the lung, where gas exchange takes place.[13] It replicates more effectively in the bronchi similar to MERS. They also proved infection and replication of the virus in the conjunctiva and gastrointestinal tract.[13] High expression of ACE2 receptors are also shown in the brush border of intestinal enterocytes[14,15] and significant titres of virus particles were detected. This could explain the subset of patients with gastrointestinal symptoms. Thus transmission through eyes and faeco-oral route serves as additional routes of infection, which are relevant for the infection prevention and control.

Apart from the detection of viral mRNA from respiratory samples, stool and conjunctiva, it was also isolated from blood, urine and saliva.[16-18] From the available evidence, active viral replication was detected in airways, alveolar epithelium, conjunctiva and gastro intestinal tract.

Duration of viral shedding

Duration of viral shedding by repeated viral cultures is warranted to ascertain the period of infectivity.

Presence of viral mRNA does not always mean active viral replication and infective potential. SARS CoV-2 RNA has been detected for 20 days or longer in one third of cohorts analysed and no association was seen between prolonged detection of viral RNA and the severity of illness.[11]

In an attempt to understand the infectivity and duration of viral shedding, live virus isolation was attempted multiple times from various clinical samples.[11,12] During the first week of symptoms, live virus was readily isolated from significant fraction of samples (16.66% of swabs and 83.33% of sputum).[12] After 8 days of symptom onset, no isolate was obtained from respiratory samples in spite of ongoing high viral loads. Generally, shedding of viral RNA from sputum outlasted the onset of symptoms. In most of the patients where symptoms wane at the end of first week, viral mRNA was detected from the upper respiratory samples and continued well into the second week and from sputum and stool, it can be detected till third week.[12] Considering the above factors, if the patients are clinically stable, home isolation can be offered to those presenting after 10 days of symptoms. Understanding this viral dynamics is important, because it was insisted that two negative swabs taken 24 hours apart were needed for discharge, but because of this intermittent shedding of viral mRNA (which need not be infectious) discharge criteria has been revised.[12]

Basic reproductive number - R_0

To calculate the degree of contagiousness or transmissibility of the coronavirus (infectivity), epidemiologists use different mathematical formulae to calculate the infectivity index. For this purpose, "basic reproductive number" R_0(pronounced as R naught or R zero) is used. It is defined as the average number of new infections generated by an infectious person in a totally naïve (uninfected) population.[19] It determines the herd immunity threshold and therefore the immunization coverage required to eliminate the disease. If R_0 is >1, the number of people infected is likely to increase and if R_0 is <1, transmission is likely to die out.

A study from Wuhan reported R_0 for SARS-CoV-2 to be 2.68 based on the imported cases from Wuhan to other cities.[20] A retrospective analysis from 12 different studies, quoted an average R_0 of 3.28 and median of 2.79.[19] In general, R_0 for COVID-19 is expected to be around 2 to 3. The difference in R_0 in various studies is because of different calculation methods and the calculations were done during various stages of the epidemic. R_0 estimates by WHO ranges from 2 and 2.5 which is higher than both SARS (1.7-1.9) and MERS (<1), suggesting the higher pandemic potential of SARS CoV -2.[21-25]

Household transmission of SARS-CoV-2

One of the important aspects of the virus transmission is its transmissibility among household members.[26] The study from Wuhan[19] enrolled 85 patients with confirmed COVID-19 and their close contacts were 155 in total.[20] Secondary attack rate was 30% among household members. Among the close contacts, infection rate was 38% for household with 1 contact, 50% for household with 2 contacts and 31% for households with 3 contacts.[20]

Another report, from analysis of cases from 20 provinces outside of Hubei in China found 1183 case clusters, out of which 64% of the clusters have been within the familial household.[27]

Analysing the outbreak in cruise ship Princess Diamond off the Japanese coast, where initially 10 people were confirmed with COVID-19 and all others were quarantined for 14 days,19% (both passengers and crew) were found to be infected when tested later.[28,29] Thus, when compared with secondary attack rate among the household contacts for MERS which is 5% and for SARS-CoV which is 10.2%. The higher secondary attack rate for SARS-CoV-2 could explain the higher speed of spread and ever increasing quantity of cases when compared to the other two corona viruses.[30,31]

Virus survival on different surfaces and environmental conditions

Though droplet transmission plays a major role in the transmission of SARS-CoV-2, aerosol (particle size <5μm) and fomite transmission is possible since the virus can remain infectious in aerosol for hours and on surfaces upto days (depending on the inoculum shed).[32] As per the recent report published, the virus remained viable in aerosol for 3 hours.[33] Viruses were applied to different objects, maintained at 21 to 23°C with 40% relative humidity over 7 days and time for significant reduction in $TCID_{50}$ (Tissue culture infectious dose) was noted. SARS-CoV-2 is more stable on plastic and stainless steel than on copper and card board. The results are shown in Table I.[34]

Stability at different temperatures

As per another recent work, SARS-CoV-2 is found to be highly sensitive to heat and at 4ºC there was only around 0.7 log unit reduction on day 14, but at 70º C, the inactivation time was reduced to 5 minutes.[33] They investigated the stability of the virus at 22ºC with a relative humidity of 65%.Virus stability on various surfaces is given in Table II.[34]

In a retrospective analysis, human coronaviruses persist for a short time at temperature of 30°C or more.[35]

Table I. Virus stability on surfaces (21 to 23°C with 40% humidity for 7 days) incubated at 21 to 23°C and 40% relative humidity over 7 days)[34]

Surfaces	Time for significant reduction in viral titres	Reduction in $TCID_{50}$
Plastic	72 hours	$10^{3.7}$ to $10^{0.6}$ per milliliter of medium
Stainless steel	48 hours	$10^{3.7}$ to $10^{0.6}$ per milliliter of medium
Copper	4 hours	No viable virus was detected after 4 hours
Card board	24 hours	No viable virus was detected after 24 hours
Aerosol	3 hours	$10^{3.5}$ to $10^{2.7}$ $TCID_{50}$ per litre of air

Thus the stability of the virus varies under different environmental conditions such as varying temperatures and humidity. In tropical countries like India with temperature nearing 40°C during summer and with average humidity of 60-70%, the viral survival on different surfaces needs to be studied.

Environmental contamination in health care premises

Many health care workers are affected by COVID-19 and hospitals are becoming the epicenter for human-to-human transmission. Recently, in a field investigation surface swabs were collected in various hospital environments and they were analyzed for the presence of SARS-CoV-2 RNA. The most contaminated objects were self service printers (20.0%), desktop/keyboard (16.8%) and door knobs (16%). Among personal protective equipments, hand sanitizer dispensers (20.3%) and gloves (15.4%) were the most contaminated objects.[32]

Frequently touched surfaces in the health care settings are therefore a potential source of virus transmission. Hence, to decrease the viral load in frequently touched surfaces in the immediate patient surroundings, appropriate disinfectants should be used. Surface disinfection with 0.1% sodium hypochlorite or 62-71% ethanol significantly reduces coronavirus infectivity on surfaces within 1 minute

Table II. Virus stability on surfaces (22°C with 65% humidity)[34]

Surfaces infectious	Time at which no virus was isolated
Printing and Tissue paper	After 3 hours
Treated wood & cloth	Day 2
Glass and bank note	Day 4
Plastic and Stainless steel	Day 7

exposure time.[32] WHO recommends 70% ethyl alcohol to disinfect small surface areas and equipment between usage such as reusable equipment (e.g.thermometer).

Immunogenicity

IgM and IgG antibodies against SARS-CoV -2 internal nucleoprotein (NP) and surface spike protein receptor binding domain (RBD) correlated with neutralising activity.[11] There are many factors which affect the antibody production including age, nutritional status, severity of the disease, certain medications or infections like HIV which suppress the immune system.[36-38] Antibody levels do not correlate with clinical course or disease severity.[11] Seroconversion occurred after 7 days in 50% and by day 14 in majority of the patients.[12] SARS-CoV-2 infections are somewhat unusual because IgM and IgG antibodies arise nearly simultaneously in serum within 2 to 3 weeks after illness onset. Thus, detection of IgM without IgG is uncommon.[39] In some patients with confirmed COVID-19 disease by RT-PCR, antibody responses were weak, late or absent.[37,38,40] Antibodies may also cross react with other human coronaviruses.[37,41,42.]

Because of the variable sensitivity and specificity, antibody testing cannot be used to diagnose COVID-19. Some clinicians make a presumptive diagnosis of recent COVID-19 disease in cases where molecular testing was negative but where there was a strong epidemiological link to COVID-19 infection and paired blood samples (acute and convalescent) showing rising antibody levels.[43] Since the appropriate antibody response happens only in the recovery phase, use of it for clinical intervention or to interrupt the disease transmission is minimal. Lastly, whether detection of antibodies could predict if an individual is immune to reinfection with the COVID-19 virus is still under debate and there is no evidence till date to support this.[43]

Tests to detect antibody responses to COVID-19 in the population will be critical to support the development

of vaccines and for understanding the extent of infection among people who are not identified through active case finding and surveillance efforts, the attack rate in the population and the infection fatality rate.[43]

Herd immunity (herd effect, community immunity, population immunity, social immunity)

Herd immunity is a form of indirect protection from infectious disease that occurs when a large percentage of a population has become immune to an infection. Immunity can be achieved either through vaccination or by contracting the infection and over a period of time natural immunity develops. When a significant proportion of the population are immune, the spread of the disease slows down or stops thereby providing a measure of protection for individuals who are not immune.

Some individuals cannot become immune because of their underlying immunodeficiency state or because of immunosuppressive medications and for this group of individuals, herd immunity offers protection. Newborn infants also cannot be vaccinated, because of their immature immune system and also the acquired antibodies from mother renders the vaccine ineffective. Once the herd immunity reaches a threshold, it helps in elimination of the disease and if the elimination was achieved globally, it results in disease eradication.

Herd immunity threshold (HIT) or herd immunity level (HIL)

When a critical proportion of the population becomes immune, the disease may no longer persist in the community.[44,45] Herd immunity threshold, in a given population, is the point where the disease reaches an endemic steady state, which means that the infection level is neither growing nor declining exponentially. The threshold can be calculated from the effective reproductive number $R_{e,}$ which can be obtained by taking the product of basic reproductive number R_0 (average number of new infections caused by an infectious case in the susceptible population) and S, the proportion of population who are susceptible to the infection. R_0 is a measure of contagiousness, so low R_0 values are associated with lower HITs, whereas higher R_0 values result in higher HITs.[45,46] For example, if the R_0 is 2, the HIT for a disease is theoretically only 50%, whereas a disease with an R_0 of 10 the theoretical HIT is 90%.[45]

The estimated R_0 and HIT of various infectious diseases is listed on Table III.

When the effective reproduction number ($R_{e)}$ is reduced to below 1 new individual per infection, the number of cases occurring in the population gradually decreases until the disease has been eliminated.[45,46,47] If the R_e increases to above 1, the disease is actively spreading through the population and infecting a larger number of people than usual.[43-46] If a population is immune in excess of that disease's HIT, the number of cases reduces at a faster rate.[47,48] So far, eradication programs based on the concept of herd immunity with reliance on vaccines have been globally successful in the case of smallpox and rinderpest, and are currently underway for poliomyelitis.[49]

Table III. Estimated R_0 and HIT of various infectious diseases[22,50-54]

Disease	R_o	HIT
Measles	12-18	92-95%
Pertussis	12-17	92-94%
Diptheria	6-7	83-86%
Rubella	6-7	83-86%
Small pox	5-7	84-86%
Polio	5-7	84-86%
Mumps	4-7	75-86%
Influenza (influenza pnademics)	1.5-1.8	33-44%
Ebola (out break in West Africa)	1.5-2.5	33-60%
SARS (2002-2004 out break)	2-5	50-80%
COVID-19 (COVID-19 pandemic)	1.4-3.9	29-74%

With regard to COVID-19 pandemic, the variables that determines the herd immunity to contain the outbreak are

i) R_0: Even though various studies quote different R_0, considering the average of 2.2, 60% of the population needs to have protective antibodies.[55]

ii) Whether the total measurable antibodies were the same as protective, virus neutralizing antibodies.[55] Even if it is protective, how long is the immunity to COVID-19 likely to last? -these questions have to be addressed.

iii) For effective herd immunity, immune response of the individuals plays an important role. Studies in COVID-19 shows that 10-20% have little or no antibody response.[56]

iv) No effective vaccine is available, with more than 100 candidate vaccines in development and few in phase 1 or phase 2 trial to assess the safety and immunogenicity.[55]

A large population based seroprevalance data is needed to ascertain the extent of population exposed and is potentially immune to the virus. With the current knowledge, there is uncertainity about the immunological correlates of protective antibodies and how much proportion of the population needs to be immune for the herd immunity effect.

Points to Remember

- ***Coronavirus family includes SARS-CoV-1, MERS and SARS-CoV-2, the currently circulating virus.***
- ***There are multiple routes of transmission for SARS-CoV-2 (respiratory, conjunctival, feco-oral routes).***
- ***Peak viral load has been demonstrated at the time of presentation.***
- ***No live virus was demonstrated from the respiratory tract after 8 days and hence infective potential gradually declines after 10 days.***
- ***Basic reproductive number R_0 is around 2 to 3, suggesting the higher pandemic potential of SARS-CoV-2.***
- ***PCR positivity does not always imply active infection, since it cannot distinguish live and dead virus.***
- ***Serology is mainly useful for epidemiological surveillance.***
- ***Herd immunity will play a role in interruption of the pandemic, but currently difficult to ascertain due to lack of information on the seroprotection levels of the population and the non-availability of vaccine.***

References

1. Englund JA, Kim Y-J, McIntosh K. Human coronaviruses, including middle east respiratory syndrome coronavirus. In: Cherry JD, Harrison GJ, Kaplan SL, Steinbach WJ, Htez PJ, editors, Feigin and Cherry's Textbook of Pediatric Infectious Diseases, 8thedn. Philadelphia: Elsevier, 2019; pp1846-1854.
2. McIntosh K, Perlman S. Coronaviruses, including Severe Acute Respiratory syndrome (SARS) and Middle East Respiratory Syndrome (MERS). In: Bennett J E, Dolin R, Blaser MJ, eds. Mandell, Douglas and Bennett's Principles and Practices of Infectious Diseases, 8th edn. Philadelphia: Elsevier, 2015; pp1928-1936.
3. World Health Organization. Summary of probable SARS cases with onset of illness from 1st November 2003 to 31st July 2004. http://www.who.int/csr/sars/country/table 2004_04_21/en/. Accessed 10th May, 2020.
4. World Health Organization. MERS-CoV summary and literature update - as of June 2013;2013. http://www.who.int/csr/disease/coronavirus_infection/update_20130620/en/. Accessed on 10th May, 2020.
5. Centers for Disease Control and Prevention. Middle East Respiratory Syndrome (MERS): Case Definitions. http://www.cdc.gov/coronavirus/mers/case-def.html. Accessed on 10th May, 2020.
6. World Health Organization. WHO statement on Coronavirus disease (COVID-19) pandemic.Out break situation. https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Accessed on 16th May, 2020.
7. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020; 395:565-574.
8. Gorbalenya AE, Baker SC, Baric R, Groot RJ de, Drosten C, Gulyaeva AA, et al. Severe acute respiratory syndrome-related coronavirus: the species and its viruses - a statement of the Coronavirus Study Group. BioRxiv 2020, https://doi.org/10.1101/2020.02.07. 937862.
9. Lauer SA, Grantz KH, Qifang Bi Q, Jones FK, Zheng Q, Meredith HR, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Internal Med https://doi.org/10.7326/M20-0504. Accessed on 30th May, 2020.
10. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579:270-273.
11. To KKW, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020; 20(5):565-574.

12. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020. https://doi.org/10.1038/s41586-020-2196-x.

13. Hui KP, Cheung MC, Perera RA, Ng KC, Bui CH, Ho JC, et al. Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. Lancet Respir Med 2020. 7thMay 2020.https://doi.org/10.1016/S2213-2600(20)30193-4.

14. Lamers, MM, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, et al. SARS-CoV-2 productively infects human gut enterocytes. Science 10.1126/science.abc1669 (2020).

15. Yu Zhao, Zixian Zhao, Yujia Wang, Yueqing Zhou, Yu Ma, Wei Zuo, et al. Single cell RNA expression profiling of ACE2 , the receptor of SARS-CoV-2. BioRxiv Jan 2020. https://doi.org/10.1101/2020.01.26.919985.

16. He Y, Wang Z, Li F, Shi Y. Public health might be endangered by possible prolonged discharge of SARS-CoV-2 in stool. J Infect 2020; 80(5):E18-E19.

17. Young BE, Ong SW, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA 2020; 323(15):1488-1494.

18. Yu Chen, Lanjuan Li. SARS-CoV-2: virus dynamics and host response. Lancet; 20(5):515-516.

19. Liu Y, Gayle A, Wilder-Smith JR A. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Travel Med 2020; 27(2):taaa021. doi:10.1093/jtm/taaa021.

20. Wang Z, Ma W, Zheng X, Wu G, Zhang R. Household transmission of SARS-CoV-2. J Infect 2020;10:28.

21. World health organization. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19). https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-finalreport.pdf. Accessed 16th Feb, 2020.

22. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020; 382(13):1199-1207.

23. Chen J. Pathogenicity and transmissibility of 2019-nCoV-A quick overview and comparison with other emerging viruses. Microbes Infect 2020; 22(2):69-71.

24. Wu JT, Leung K, Leung GM. Now casting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. Lancet 2020; 395(10225):689-697.

25. Liu T, Hu J, Kang M, Lin L, Zhong H, Xiao J, et al. Transmission dynamics of 2019 novel coronavirus (2019-nCoV). BioRxiv. January 2020. https:// doi.org/10.1101/2020.01.25.919787.

26. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneu- monia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020; 395(10223):514-523.

27. Wu Z, McGoogan JM. Characteristics of and important lessons from the coron- avirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese center for disease control and prevention. JAMA 2020 Feb 24.

28. Rocklöv J, Sjödin H, Wilder-Smith A. COVID-19 outbreak on the Diamond Princess cruise ship: estimating the epidemic potential and effectiveness of public health countermeasures. J Travel Med 2020 Feb 28.

29. NHK. Confirmed 947 infection of 2019 novel coronavirus in Japan. www3.nhk. or.jp/news/special/coronavirus/. Date last updated: March 1 2020.

30. Drosten C, Meyer B, Müller MA, Corman VM, Al-Masri M, Hossain R, et al. Transmission of MERS-coronavirus in household contacts. N Engl J Med 2014; 371(9):828-835.

31. Wilson-Clark SD, Deeks SL, Gournis E, Hay K, Bondy S, Kennedy E, et al. House- hold transmission of SARS, 2003. CMAJ 2006; 175(10):1219-1223.

32. Ye G, Lin H, Chen L, Wang S, Zeng Z, Wang W, et al. Environmental contamination of the SARS-CoV-2 in healthcare premises: An urgent call for protection for healthcare workers. MedRxiv. https://doi.org/10.1101/2020.03.11.20034546.

33. Doremalen NV, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and Surface Stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 2020; 382:1564-1567.

34. Chin A, Chu J, Perera M, Hui K, Yen HL, Chan M, et al. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe 2020;1(1):E10.

35. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of corona viruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect 2020; 104(3): 246-251.

36. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Medxriv. Clin Infect Dis 2020. ciaa344, https://doi.org/10.1093/cid/ciaa344.

37. Okba NA, Muller MA, Wentao Li, Wang C, GeurtsvanKessel CH, Corman VM, et al. SARS-COV-2 specific antibody responses in COVID-19 patients. Medxrix. 2020; https://www.medrxiv.org/content/10.1101/2020.03.18.20038059. Accessed on 31st May, 2020.

38. Gorse GJ, Donovan MM, Patel GB. Antibodies to coronaviruses are higher in older compared with young adults and binding antibodies are more sensitive than neutralizing antibodies identifying coronavirus - associated illnesses. J Med Virol 2020; 92(5):512-517.

39. Interim Guidelines for COVID-19 Antibody Testing | CDC www.cdc.gov/coronavirus/2019-ncov/lab/resources/ Accessed on 31st May, 2020.
40. Lin D, Liu L, Zhang M, Hu Y, Yang Q, Guo J, et al. Evaluation of serological tests in the diagnosis of 2019 novel coronavirus (SARS-CoV-2) infections during the COVID-19 outbreak. Medxriv. 2020; https://doi.org/10.1101/2020.03.27.20045153.
41. Wang N, Li SY, Yang XL, Huang HM, Zhang YJ, Guo H, et al. Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. Virol Sin 2018; 33(1):104-107.
42. Che XY, Qiu LW, Liao ZY, Wang YD, Wen K, Pan YX, et al. Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43. J Infect Dis 2005; 191(12):2033-2037.
43. WHO. Advice on the use of point-of-care immunodiagnostic tests for COVID-19: Scientific brief, April 2020. https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-COVID-19" https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-COVID-19. (Accessed on 8th April, 2020).
44. Somerville M, Kumaran K, Anderson R. Public Health and Epidemiology at a Glance. 2nd edn; John Wiley & Sons; 2012; pp58-59.
45. Rodpothong P, Auewarakul P. "Viral evolution and transmission effectiveness". World J Virol 2012; 1(5): 131-134.
46. Perisic A, Bauch CT. "Social contact networks and disease eradicability under voluntary vaccination". PLoS Comput Biol 2009; 5(2):e1000280.
47. Dabbaghian V, Mago VK. Theories and Simulations of Complex Social Systems. Springer 2013; pp134-135.
48. Garnett GP. "Role of Herd Immunity in Determining the Effect of Vaccines against Sexually Transmitted Disease". The Journal of Infectious Diseases 2005; 191(1):S97-106.
49. Fine P, Eames K, Heymann DL. "Herd immunity": A rough guide". Clin Infect Dis 2011; 52 (7):911-916.
50. Unless noted, R_0 values are from: History and Epidemiology of Global Smallpox Eradication Archived 2017-03-17 at the Wayback Machine From the training course titled "Smallpox: Disease, Prevention, and Intervention". The Centers for Disease Control and Prevention and the World Health Organization. Slide 17.
51. Biggerstaff M, Cauchemez S, Reed C, Gambhir M, Finelli L. Estimates of the reproduction number for seasonal, pandemic and zoonotic influenza: A systematic review of the literature. BMC Infect Dis 2014;14:480.
52. Wallinga J, Teunis P. "Different epidemic curves for severe acute respiratory syndrome reveal similar impacts of control measures". Am Jl of Epidemiol 2004; 160(6): 509-516.
53. Riou J, Althaus CL "Pattern of early human-to-human transmission of Wuhan 2019 novel coronavirus (2019-nCoV), December 2019 to January 2020". Euro Surveill 2020; 25 (4):2000058.
54. Althaus CL. "Estimating the Reproduction Number of Ebola Virus (EBOV) During the 2014 Outbreak in West Africa". PLOS Currents 2014; 6.
55. Altmann DM, Douek DC, Boyton RJ. What policy makers need to know about COVID-19 protective immunity. The Lancet 2020; 395(10236):p1527-1529.
56. Tan W, Lu Y, Zhang J, Wang J, Dan Y, Tan Z, et al. Viral kinetics and antibody responses in patients with COVID-19. medRxiv2020; https://doi.org/ 10.1101/2020.03.24.20042382.

CLIPPINGS

Seroprevalence of novel coronavirus disease (COVID-19) in Kobe, Japan.

A cross-sectional serologic testing for SARS-CoV-2 antibody was done on 1,000 samples from patients at outpatient settings who visited the clinic from March 31 to April 7, 2020, stratified by the decade of age and sex. There were 33 positive IgG among 1,000 serum samples (3.3%, 95%CI: 2.3-4.6%). By applying this figure to the census of Kobe City (population: 1,518,870), it is estimated that the number of people with positive IgG be 50,123 (95%CI: 34,934-69,868). Age and sex adjusted prevalence of positivity was calculated 2.7% (95%CI: 1.8-3.9%) and the estimated number of people with positive IgG was 40,999 (95% CI: 27,333-59,221). These numbers were 396 to 858 fold more than confirmed cases with PCR testing in Kobe City.

Conclusions: This cross-sectional serological study suggests that the number of people with seropositive for SARS-CoV-2 infection in Kobe, Japan is far more than the confirmed cases by PCR testing.

Doi A, Iwata K, Kuroda H, Hasuike T, Nasu S, Kanda A. Seroprevalence of novel coronavirus disease (COVID-19) in Kobe, Japan.medRxiv Preprint. May 1, 2020. https://doi.org/10.1101/2020.04.26.20079822.