A biography of coronaviruses from IBV to SARS-CoV-2, with their evolutionary paradigms and pharmacological challenges

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ABSTRACT
Coronaviruses (CoVs) are a family (Coronaviridae) of viruses that cause respiratory disorders in birds and mammals. They were originally discovered as infectious bronchitis virus (IBV) of chickens in the early 20th century. A group of related viruses subsequently discovered from mice and humans led to the collective naming as coronaviruses, as they were all characterized by a solar corona-like ring on their surface, called the spikes. The first known human CoVs were among viruses that cause common cold and considered as modest threats to human health. But the emergence of zoonotic CoVs such as severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and Severe acute respiratory syndrome-related coronavirus (SARS-CoV-2) during every recent past decade resulted in pandemics with loss of human lives. With no vaccine to prevent, and no drug to treat the infections, the miniscule viruses evolve into behemoths of plagues. SARS-CoV-2 with its infection, COVID-19, is particularly rampant and malicious and is bound to cause colossal impacts not only on human health but also on global economy. Understanding of their evolutionary strategies and pathogenic adaptions had given us fair warnings. Yet, the world was ill-prepared. This article highlights the scientific messages that could have mitigated the COVID-19 pandemic, the evolutionary mechanisms in SARS-CoV-2 and related CoVs that bely drug and vaccine development, and above all, the possible epidemics in the future.

INTRODUCTION
Coronaviruses (CoVs) are a family of zoonotic viruses that normally infect birds and mammals. They are responsible for a wide range of diseases including cough, fever, pneumonia, enteritis, encephalitis, and hepatitis, many of which are often proved to be fatal (Chen et al., 2020). Their virulent nature has been a serious threat to animal farming and wildlife (Miłek and Blicharz-Domańska, 2018). But their adaptive infections to humans in recent years have proven to be of utmost dire consequences, not only on medical perspectives, but also on the socio-economic conditions. The first known human CoVs, HCoV-229E and HCoV-OC43, discovered in the 60s were safely cast aside as agents of nothing more than common colds. But it was a preposterous idea, as novel human CoVs arrived on the scene, bringing about devastating blow to global health and economy.

The supreme virulence coupled with infectivity of CoVs among all viruses in humans had been unleashed in each of the past three decades. Hitherto unknown to science, severe acute respira-
Coronavirus (SARS-CoV) emerged in 2002/2003 with shocking audacity as the first deadly CoV in humans. Its infection, the severe acute respiratory syndrome (SARS) became an epidemic that killed 774 people in one clean sweep. MERS-CoV followed after ten years and with it the Middle East respiratory syndrome (MERS) caused fatality of 291 people. The third in the panel appeared with a global pandemic of an epic proportion in 2020. With its officially christened species name SARS-CoV-2 or Severe acute respiratory syndrome -related coronavirus (Gorbalenya et al., 2020), its rampant infection is enumerated in millions, and mortality in hundreds of thousands, and still counting as of writing this paper.

CoVs (family Coronaviridae) are monophyletic viruses bearing positive-sense, single-stranded RNA, or (+) ssRNA, and are categorised into four genera, namely Alphacoronavirus, Betacoronavirus, Deltacoronavirus, and Gammacoronavirus. As of 2020, there are 39 species of CoVs (Gorbalenya et al., 2020). They comprise the largest member in the order Nidovirales, which contains three families Coronaviridae, Arteriviridae, and Roniviridae. Betacoronavirus is further divided into four subgenera simply named group A, B, C, and D (Wu et al., 2020). Human CoVs fall under the genera Alphacoronavirus and Betacoronavirus (Table 1).

It is worthwhile to mention that there are few coronaviruses which are already described, especially from birds, which are yet to be assigned genus or, perhaps, higher taxa. Regardless of their diversity and host range, they are able to cause mild to severe respiratory and related diseases. There is no drug or vaccine for the viruses. This is mainly because of how CoVs evade immune reactions in complex cellular mechanisms (Chen et al., 2020). They invade the primary immune cells, macrophages and thereby downplaying cytokine signaling process that would have otherwise activated the production of type I interferons (IFN-α/β). They possess many IFN antagonists to kick start a chain of inflammatory reaction resulting in the development of the symptoms of infection. However, it is not yet entirely certain how they, in particular SARS-CoV-2, invade the immune cells and exert varying degree of clinical symptoms. In this article, I attempt to explain this pathogenic mechanism in the light of historical account if the discoveries and evolution of the CoVs.

**Discovery of the Coronaviruses**

**The chicken virus (infectious bronchitis virus)**

The coronavirus infection, but not the virus or the name, was first described by American veterinarians Arthur Frederick Schalk and Merle C. Fawn at the North Dakota Agricultural College. Their report in 1931 indicates that 2-day-old to 3-week-old chickens readily fell prey to hitherto unknown respiratory disease. The chickens easily developed shortness of breath and lethargy, and the infection was highly fatal with mortality as high as 90%. The infection was extremely contagious through direct contact or experimental transfer of the bronchial exudates of infected individual to healthy chickens. Schalk and Fawn referred to the new disease as “an apparently new respiratory disease of baby chicks” (Schalk, 1931).

Another line of discoveries was a bit more confusing. In the early 1920s, it had been reported that there was a new respiratory disease of chickens in US and Canada (Gwatkin, 1925). The disease was variously called infectious laryngotracheitis or infectious bronchitis. The infection was detected in the exudates of the larynx and trachea of infected chickens. Bacteria were ruled out as the pathogens, because bacteria isolated from infected chicken did not produce the symptoms. Robert Graham, Frank Thorp Jr. and William Arthur James at the Illinois Agricultural Experiment Station reported in 1930 that there were actually two distinct but overlapping symptoms, which they referred to as acute and subacute forms that could be easily distinguished, and that the acute form was predictably caused by a filterable virus (Graham et al., 1930).

Jerry Raymond Beach at the University of California quickly solved one of the problems. He identified and isolated the causative agent as a filterable virus in 1930 (Beach, 1877). He found that the virus could infect the respiratory tract as well as the spleen and livers. The next year he experimentally demonstrated that it was the virus alone that produced the symptoms, even though other pathogens were present. His novel virus was later called infectious laryngotracheitis virus (ILV), or more specifically gallid alpha herpesvirus 1. It is not a coronavirus, but a member of the order Herpesvirales.

This side stepping in the narrative is crucial because the CoVs emerged right in the turmoil of the confusion. Leland David Bushnell and Carl Alfred Brandy at the Kansas Agricultural Experiment Station, Manhattan, had been investigating since 1930 an outbreak of what was called “gasp ing disease” among new-born chickens in Kansas. They reported the cases as “laryngotracheitis in chicks”. They asserted that the disease was caused by “a filter-passing virus” (Bushnell and Brandy, 1933). They were quite clueless about their discovery of a new virus, the very causative agent of Schalk and Fawn’s “new respiratory disease of baby chicks”. In 1936,
Table 1: List of medically important species of coronaviruses

<table>
<thead>
<tr>
<th>Species</th>
<th>Genus</th>
<th>Host</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human CoV-229E</td>
<td>Alphacoronavirus</td>
<td>Human</td>
<td>Common cold</td>
</tr>
<tr>
<td>Human CoV-NL63</td>
<td>Alphacoronavirus</td>
<td>Human</td>
<td>Common cold</td>
</tr>
<tr>
<td>PRCV/ISU-1</td>
<td>Alphacoronavirus</td>
<td>Pig</td>
<td>Mild respiratory tract infections</td>
</tr>
<tr>
<td>TGEV/PUR46-MAD</td>
<td>Alphacoronavirus</td>
<td>Pig</td>
<td>Diarrhoea, with 100% mortality in piglets</td>
</tr>
<tr>
<td>PEDV/ZJU-G1-2013</td>
<td>Alphacoronavirus</td>
<td>Pig</td>
<td>Severe watery diarrhoea</td>
</tr>
<tr>
<td>SeACoV-CH/GD-01</td>
<td>Alphacoronavirus</td>
<td>Pig</td>
<td>Severe and acute diarrhoea and acute vomiting</td>
</tr>
<tr>
<td>HKU2r-CoV</td>
<td>Alphacoronavirus</td>
<td>Pig</td>
<td>Severe and acute diarrhoea</td>
</tr>
<tr>
<td>Canine CoV/TU336/F/2008</td>
<td>Alphacoronavirus</td>
<td>Dog</td>
<td>Mild clinical signs, diarrhoea</td>
</tr>
<tr>
<td>Feline infectious peritonitis virus</td>
<td>Alphacoronavirus</td>
<td>Cat</td>
<td>Fever, vasculitis, and serositis, with or without effusions</td>
</tr>
<tr>
<td>Human CoV-HKU1</td>
<td>Betacoronavirus</td>
<td>Human</td>
<td>Pneumonia/common cold</td>
</tr>
<tr>
<td>Human CoV-OC43</td>
<td>Betacoronavirus</td>
<td>Human</td>
<td>Common cold</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>Betacoronavirus</td>
<td>Human</td>
<td>Severe acute respiratory syndrome, 37% mortality rate</td>
</tr>
<tr>
<td>SARS-CoV</td>
<td>Betacoronavirus</td>
<td>Human</td>
<td>Severe acute respiratory syndrome</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Betacoronavirus</td>
<td>Human</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>Bovine CoV/ENT</td>
<td>Betacoronavirus</td>
<td>Cow</td>
<td>Diarrhoea</td>
</tr>
<tr>
<td>Equine CoV/Obihiro12-1</td>
<td>Betacoronavirus</td>
<td>Horse</td>
<td>Fever, anorexia, leucopenia</td>
</tr>
<tr>
<td>MHV-A59</td>
<td>Betacoronavirus</td>
<td>Mouse</td>
<td>Acute pneumonia and severe lung injuries</td>
</tr>
<tr>
<td>Beluga Whale CoV/SW1</td>
<td>Gammacoronavirus</td>
<td>Whale</td>
<td>Pulmonary disease, terminal acute liver failure</td>
</tr>
<tr>
<td>IBV</td>
<td>Gammacoronavirus</td>
<td>Chicken</td>
<td>Severe respiratory disease</td>
</tr>
<tr>
<td>Bulbul coronavirus HKU11</td>
<td>Deltacoronavirus</td>
<td>Bulbul</td>
<td>Respiratory disease</td>
</tr>
<tr>
<td>Sparrow coronavirus HKU17</td>
<td>Deltacoronavirus</td>
<td>Sparrow</td>
<td>Respiratory disease</td>
</tr>
</tbody>
</table>
Beach reanalysed Bushnell and Brandly’s findings and experimentally confirmed that infectious laryngotracheitis and infectious bronchitis were distinct diseases, and that the pathogenic viruses were also unique for each (Beach and Schalm, 1936). Bushnell and Brandly had unknowingly discovered the first coronavirus, which was eventually named infectious bronchitis virus (IBV).

The mouse viruses

Since the early 1940s, brain disease (murine encephalomyelitis) was fairly common in mice in which neurons (specifically the myelin sheath) were spontaneously damaged. Theiler’s viruses (Theiler’s murine encephalomyelitis viruses) were identified as the main aetiological factors. But there was a different story for the same disease. Francis Sargent Cheevers, Joan B. Daniels, Max Pappenheimer Jr., and Orville T. Bailey colleagues examined two laboratory mice that had undergone flaccid paralysis on 14 August 1947 at the Department of Bacteriology and Immunology, Harvard Medical School in Boston. The mice showed no signs of illness or diarrhoea, normally seen in murine encephalomyelitis. The virus called JHM was isolated from all vital organs, the liver, spleen, lungs, and kidneys from infected mice (Cheever et al., 1949). The virus also caused necrosis of liver, indicating hepatitis.

There was another episode of an outbreak of fatal infection among the breeding stock of mice (Parkes or P strains) at the National Institute for Medical Research, Mill Hill, London, in the autumn of 1950. Alan Watson Gledhill and Christopher Howard Andrewes identified the disease as murine hepatitis, and the pathogen as a new type of virus, which they named mouse hepatitis virus (MHV) (Gledhill and Andrewes, 1951). The relationship between IBV, JHM and MHC was not apparent at all. The first clue
was when John A. Morris at the National Institutes of Health, Bethesda, reported the discovery of a new murine virus, named H747, from Japan in 1959. Morris found that H747 was antigenically similar to JHM and MHV and concluded that they belong to the same group of viruses (Morris, 1959). With the advancement of electron microscopy, images of IBV and MHV revealed that the viruses were structurally similar. They appeared to be the same virus.

The human viruses

David Arthur John Tyrrell working at the Common Cold Research Unit (CCRU) of the British Medical Research Council at Salisbury was assigned to study the aetiology of common cold. His team had established that common cold viruses could be categorised into M strain, those that can be cultured both in human-embryo-kidney cell culture and monkey-embryo-kidney cell culture, and H strain, those that can be maintained only in human-embryo-kidney cell culture. But samples (throat and nasal swabs) collected from schoolboys during 1960 to 1961 could not be cultured by their conventional methods, implying that the viruses were neither M nor H strains. One sample, designated B814, obtained from a boy was exceptionally infectious in healthy volunteers. They identified the virus in 1965 (Tyrrell and Bynoe, 1965). It was the first human CoV discovered.

Dorothy Hamre and John J. Procknow at the Department of Medicine, University of Chicago, collected in 1962 six samples from medical students who were suffering from upper respiratory tract infection. The
samples, designated 229E, were immunologically different from all known viruses. They reported the new virus in 1966 (Hamre and Procknow, 1966). They later established that 229E was ether-sensitive ribonucleic acid virus. Under electron microscopy, IBV, MHV, B814 and 229E appeared identical in all respects. A collective new name for IBV, JHH, MHV, B814 and 229E was created in 1968 as coronaviruses, as a reminiscence of the circular surface projection to solar corona characteristic of the viruses (Almeida et al., 1968).

**Structural Identity and Pharmacological Targets**

It is rather disappointing that after almost a century of their discovery, and half a century of establishing their structure, we still do not have the pharmaceutical remedy or vaccine against them. The problem lies in the complex structural organisations. Being pleomorphic entities, CoVs are highly variable in structure and with that differ in their infectivity and pathogenicity. But they do share basic structural organisation. A typical CoV is spherical in shape and measures approximately 120 to 160 nm. A typical CoV is entirely covered by a proteinaceous envelope, made up of the membrane (M) protein, the envelope (E) protein, and the spike (S) protein (Figure 1). The genetic material, RNA molecule, lies internally and is covered with the nucleocapsid (N) protein.

The main framework of the envelope is made up of M protein, a type III membrane protein. It is remarkably thick, at 7.8 nm, for a biological membrane. It has three domains: a short N terminal ectodomain, a triple-spanning transmembrane domain, and a C-terminal endodomain. The embedded portion of the proteins, called C-terminal domain, appears to form a matrix-like lattice, which contribute to the extra-thickness. The M proteins are indispensable in the life cycle of the virus such as during assembly, budding, envelope formation, and pathogenesis. The E proteins are minor proteins dispersed...
among the M proteins. They have (at least) two distinct domains namely transmembrane domain and extramembrane C-terminal domain, which form ion channels and act as membrane permeabilizing proteins (viroporins) (Chen et al., 2020). Both the M and E proteins are highly conserved across species, and thus, would not be of interest for drug targets.

The most interesting structural proteins as potential drug target are the S proteins. They are the proteins that form the corona of the CoVs. They are type I glycoproteins which exist in two subunits, S1 and S2 (Figure 1). The two subunits remain noncovalently linked as they are exposed on the viral surface, until they attach on the host cell membrane. Three S1 subunits and two S2 subunit constitute a single spike, which is normally 15 to 20 nm in diameter. The S1 subunits are the receptor-binding sites of CoVs, and contain a signal peptide, followed by an N-terminal domain (NTD) and receptor-binding domain (RBD). Whereas the S2 proteins are responsible for fusion of the viral envelope with that of the host cell, and contain conserved fusion peptide (FP), heptad repeat (HR) 1 and 2, transmembrane domain (TM), and cytoplasmic domain (CP) (Lu et al., 2020). As such S proteins are responsible for the pathogenicity, host specificity and immunogenic properties of the virus, and are therefore the main focus for drug target and vaccine development.

The prime target of interest on the S protein would be the receptor-binding domains (RBDs) on S1 subunits (Figure 2). The RBD is composed of N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD) that are able to recognise either carbohydrates or proteins on the host cell surface. For examples, the bovine coronavirus (BCoV) NTD recognizes 5'-N-acetyl-9-O-acetylatedneuraminic acid on the host cell. MHV NTD binds to carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1). Human CoV HKU1 NTD binds to O-acetylated sialic acid as an attachment receptor determinant and to hemagglutinin-esterase protein as a receptor-destroying enzyme (Lu et al., 2020).

Species of Betacoronavirus normally use CTD, which are more versatile in recognizing different protein receptors such as angiotensin-converting enzyme 2 (ACE2), aminopeptidase N (APN), and dipeptidyl peptidase 4 (DPP4). For this reason, CoVs using CTD can invade a variety of cells and tissues. For instance, SARS-CoV and SARS-CoV-2 bind to ACE2, which are expressed on the cells of adipose tissue, gall bladder, heart, intestine, kidney, ovary, respiratory tract, testis, and thyroid glands. Regardless of the species, the core RBD is only six amino acid sequence and is highly immensely different between species. For example, the core RBD in human CoV-NL63 is Lys-353, Phe-493, Tyr-498, Ser-535, Trp-541, and Trp-585; in SARS-CoV it is Tyr-442, Leu-472, Asn-479, Asp-480, Thr-487 and Tyr-4911; while in SARS-CoV-2 it is Leu-455, Phe-486, Gln-493, Ser-494, Asn-501 and Tyr-505 (Andersen et al., 2020). The implication of these receptor-binding variations is that drugs and vaccines would have to be very specific either for the CoV RBD or the host cell’s receptor.

**SARS-CoV-2**

**Emergence and evolutionary adaptations**

SARS-CoV-2 was discovered upon an investigation that caused severe pneumonia of unknown aetiology in clusters of 27 people following reports of several health centres in Wuhan, China, on 31 December 2019. Identification of the virus as a novel type led to the naming “WH-Human 1” on 3 February 2020 after the city of its origin. The World Health Organization adopted “2019 novel coronavirus” or “2019-nCoV” for the virus and “coronavirus disease 2019” (COVID-19) as the name of the disease on 11 February 2020 (Tang et al., 2020). The International Committee on Taxonomy of Viruses (ICTV) finally announced the official species name as “Severe acute respiratory syndrome-related coronavirus” with the abbreviation as “SARS-CoV-2” on 2 March 2020 (Gorbalenya et al., 2020). The new name was not entirely without criticism. The novel virus is not most closely related to SAR-CoV, does not cause severe acute respiratory syndrome in a strict sense but pneumonia, and the new name had little bearing on the approved name of the disease.

SARS-CoV-2 is in fact most closely related to bat SARS-like-CoVs, particularly SL-CoV-RaTG13. Its genome exhibits 96% nucleotide similarity to SL-CoV-RaTG13 of the Chinese horseshoe bat Rhinolophus sinicus, the fact of which itself will show that the two are technically the same species. Its distant bat CoV relatives are SL-CoVZC45 with which it shares 87 to 89% genome identity (Tang et al., 2020), and SARS-like-CoVZXC21 with which it shares 89% similarity (Chan et al., 2020). These genetic similarities indicate that the virus is member of the genus Betacoronavirus. Specifically, it belongs to Betacoronavirus lineage B, under the subgenus Sabecovirus. In terms of protein similarity, SARS-CoV-1 is most identical (91.1%) to SL-CoVZXC21 (MG772934) of bat, while it is only 77.1% similarity with SARS-CoV. The S2 subunit of the spike (S) proteins in SARS-CoVs and SARS-like CoVs (SL-CoVZXC21 and ZC45) is highly conserved with 99% identity across the species. But the S protein in SARS-CoV-1 and SARS-CoV-2 share an overall 89.8% amino acid sequence
identity, while the core receptor-binding domain are more or less identical (Chan et al., 2020). This indicates that both viruses use ACE2 as the receptor to invade different human cells.

But a remarkable feature is that SARS-CoV and SARS-CoV-2 are not genetically related. They share only an average of 81% (79 to 82%) nucleotide sequence similarity (Tang et al., 2020). This is a keystone in the technical argument for assigning them into the same species. A more comprehensive genome sequencing shows that SARS-CoV exhibits 88% similarity to bat-SL-CoVZC45 and bat-SL-CoVZXC21, and only 79% similarity to SARS-CoV and 50% similarity to MERS-CoV (Lu et al., 2020). The implication is that if the species criterion of 90% genetic similarity as laid down by ICTV is to be followed, SARS-CoV-2 is undoubtedly member of bat coronaviruses, and not of humans. Why the ICTV breaks the very rule of its own creation is not fully justified.

An important question that is yet to be resolved is the extremely contagious nature of SARS-CoV-2. Looking back at the first SARS-CoV and MERS-CoV, it is easy to understand that they were efficiently causing epidemics but had limited capacity for human-to-human transmission. They vanished within some months of the outbreaks, which suggests that they had poor evolutionary adaptation for effective infectivity between humans. But SARS-CoV-2 is very different. Regardless of the lack of direct molecular evidence on the cellular invasion mechanism, the evolutionary signatures are being unfolded. The S1 subunit of SARS-CoV-2 S protein shares only 70% similarity to those of other SAR-CoVs (Chan et al., 2020). It exhibits 10- to 20-fold higher affinity to ACE2 than that of SARS-CoV. In addition, SARS-CoV-2 contains a unique S1/S2 furin-recognizable site which likely contributes to an efficient process of cell fusion. Unlike in other CoVs, SARS-CoV-2 infected cells display an ability to attract the adjacent uninfected cells. Consequently, many cells fuse together as multinucleated and massive cellular structure called syncytium. Other mutations in N proteins could also strengthen the stability of cellular fusion and increase the infectivity (Benvenuto et al., 2020). These altered protein architectures would increase the rates of cellular invasion and destructions.

It is precisely because of the complexity and our incomplete understanding of the pathogenicity and infectivity of SARS-CoV-2 that the notion of the virus as part of a biological weapon experiment cannot be a reasonable conjecture. We now at least understand that CoVs are susceptible to genetic changes for adaptation, and thereby often geared for cross-species transmissions. Phylogenetic analysis showed that variation among SL-CoVs is due to genetic recombination and that SARS-CoV was a product of such genetic reshuffling of SL-CoVs. SARS-CoV in civets came from SL-CoV in 2008, i.e. 4.08 years before the SARS outbreak (Hon et al., 2008). As such, SARS-CoV-2 with 380 amino acid substitutions amassed from other CoVs (Wu et al., 2020) is of all for spill-over infection to different hosts. The capacity of SARS-CoV-2 to cross multi-spectrum hosts was confirmed by the fact that cats, dogs and a four-year-old female Malayan tiger, named Nadia, at the Bronx Zoo New York City, have been infected by the virus. Cats are especially highly susceptible to airborne infection with the virus. As is true in all pathogens, adaptation in new host always starts with most detrimental effects; thus, it is natural to expect outbreak worse than the previous.

Caveat for the pandemic

The first SARS outbreak in 2002–2003 unfolded a succession of alarming scientific accounts. The masked palm civets (Paguma larvata) and possibly raccoon dog (Nyctereutes procyonoides) sold at the Dongmen Market in Shenzhen, Guangdong Province, were identified as the likely sources of the virus. Genetic and serological tests confirmed that the animals and the people connected to the market had the same novel viral infection. After the epidemic, there was an isolated outbreak late in 2003. Four people were diagnosed with pneumonia between 16 December 2003 and 8 January 2004. Two were waitresses at a local restaurant in Guangzhou, Guangdong Province, and the others were customers. They were all confirmed of SARS-CoV infection. Genetic analyses revealed that they and six masked palm civets kept in cages inside the restaurant were infected with SARS-CoV. The Guangdong provincial authority immediately banned hunting, trade, and transportation of all wild animals. Animals that could harbour the virus in farms and in animal food markets were slaughtered. Farmed civets were quarantined. The infection never reappeared (Shi and Hu, 2008). The masked palm civets were regarded as the source of the infection as in the original epidemic.

Extensive surveys indicated that SARS-CoV was not that prevalent in masked palm civets, farmed and wild. There must be a different source. By then it was an established fact that bats were the veritable hot spots of human-pathogenic viruses including Ebola virus, Nipah virus, Lyssavirus (rabies virus), Hendra virus, and St. Louis encephalitis virus. The first investigation on bats as a source of SARS-CoV in China led to the discovery of sev-
eral novel bat CoVs from *Miniopterus magnater*, *Miniopterus pusillus*, and *Miniopterus schreibersii*; but not SARS-CoV. These bat CoVs share only 41–62% nucleotide sequence identities to SARS-CoV and belong to *Alphacoronavirus*. But contemporaneous surveys revealed that the Chinese horseshoe bat (*Rhinolophus sinicus*) are a storehouse of several SARS-like coronaviruses (SL-CoVs) (*Lau et al., 2005*). These discoveries foretold that bats and their viruses were forebears of epidemics.

Wendong Li and colleagues, in their report in 2005, concluded that there was a possibility of SARS-CoV variants crossing the species barrier and infect humans (*Menachery et al., 2015*). To make the warning more lucid, SHC014-CoV in Chinese horseshoe bat was not only related to human SARS-CoV, but also binds to ACE2, implying that the virus could infect humans. Cell cultures using mice and human tracheal tissues verified the infectivity. In 2018, it was reported that human infection with bat CoVs had already happened among people in Jinning County, Yunnan Province, China, as shown by serological tests. An additional test in some inhabitants of Wuhan in Hubei Province also tested positive (*Menachery et al., 2016*). Yi Fan and colleagues at the Chinese Academy of Sciences espoused more pressing statements that bat CoVs would “re-emerge to cause the next disease outbreak. In this regard, China is a likely hotspot,” and that the infection could “lead to the generation of potential pandemic viruses” (*Fan et al., 2019*). They would not have been much surprised as SARS-CoV-2 emerged in December 2019.

**Evolutionary Retrospection and Perspectives**

It is no longer feasible for retrospective immunological tests from blood samples to infer when or how the first infection with SARS-CoV-2 happened as the epidemic was swift and global. What is so far definitive is that SARS-CoV-2 probably originated from bats. But in the absence of coherent evidence that the virus is actually present in bats, and the difficulty or impossibility to reconstruct the initial human infection, the most we can do to explain is to extrapolate how and where the virus evolved in the first place. I shall consider here various scenarios of biological adaptations (Figure 3), and disregard notions such as biological weapon as the probable origin of the virus.

The first theory with a sound support is that the virus originated or at least came from Malayan pangolins (*Manis javanica*). The idea is evidence by the discovery of SARS-CoV-2 like CoVs in these mammals that show 85.5 to 92.4% nucleotide similarity to SARS-CoV-2. The RBD similarity is particularly high with 97.4% amino acid similarity with that of SARS-COV-2. Another genome analysis indicated 91.02% similarity. But significant differences were noted including the absence of cleavage site for furin proteases in pangolin CoV (?). An important point is that SARS-CoV-2 is more closely related to SL-COVRaTG13 and other bat CoVs than it is to the pangolin CoV. The similarity of RBD, as short amino acid sequence, between SARS-COV-2 and pangolin CoV could be due to convergent evolution. Key structural variations are also seen especially in the S protein. These analyses imply that human could not have acquired the virus from pangolins, or that pangolins could not even be the intermediate hosts. The pangolin CoV could just be a spill-over and cross transmission of bat SL-CoV.

The second scenario is bats as the direct source of SARS-CoV-2, in which the necessary features of infectivity and pathogenicity developed (?). The fact SARS-CoV-2 is most closely related to bat SARS-like CoVs, particularly SL-CoV-RaTG13, is a persuasive indication. Genomes with 96% nucleotide similarity is no small measure. It suggests that SARS-CoV-2 by default is a bat virus. However, SARS-CoV-2, or virus with the unique features of SARS-CoV-2 are not (yet) found in bats. This is very relevant because if SARS-CoV-2 is a genuine bat virus, we would expect many bats to carry the virus, and that there would be clear indication of transmission to humans in several occasions. But these are not so. Survey of animal specimens in the markets yield negative possibility such zoonotic multiple transmission.

The third and most parsimonious theory is that SARS-CoV-2 is an exclusively human virus, originally evolved from bat SL-CoV. This supposition also posits that SARS-CoV-2 did not undergo mutation in the bat host to acquire zoonotic mechanism for infectivity to human host. It is very likely that virus was in its chiropteran-native state caused nothing more than mild symptoms, if at all. This is a credible premise because Bat CoVs are remarkably innocuous in their hosts. The entire genetic changes happened in human host. It is already known that some bat SL-CoV could bind to human ACE2 (*Menachery et al., 2015, 2016*). The initial human encounter would be rather benign. But as the virus was exposed to selective pressure under the bombardment of the human immune cells, the crucial variation leading to pathogenicity and high-affinity binding to human ACE2 must have arisen by mutation through natural selection after the infection was achieved (*Andersen et al., 2020*).

Even though the data is not exhaustive, there has been evidently opportunistic infections of humans.
with bat SL-CoVs. Serological data from the inhabitants of Jinning County, Yunnan province, China, between 2015 and 2018 indicated steady but low level of human infections (Wang et al., 2018). Viral examination specific for SARS-CoV-2 genomic traces in human faecal samples provides further testimonies of infection prior to the emergence of the epidemic. Stool samples collected from China before April 2019 indicated SARS-CoV-2 genomic traces in 6 out of 26 samples (Rampelli et al., 2020). This testifies that transmission of the virus to humans had occurred before April 2019 fortifying the proposition that the virus was initially quite harmless, and that it had a great deal of time to undergo mutations. The ability of the virus to undergo rapid evolutionary changes is indubitable as genomic variations in different infected people showed. Population analysis revealed that the virus has been circulating in human in two distinct genomic types, named L and S. The S lineage is older and more benign with the prevalence rate of 30%; whereas the L type is the more virulent and more recent, with 70% prevalence (Tang et al., 2020). This is a further support to the human-only speciation hypothesis.

CONCLUSIONS

Coronaviruses have been with us for a century and pose serious threats to animal and human welfare. They are evolving on unprecedented scale and becoming more menacing in each epidemic. SARS-CoV-2 as the most recent evolutionary product, is a true global menace, affecting every facet of human life from health, economy, education to politics. But careful examination of scientific literature shows that we have been warned, the epidemic has been foreshadowed by previous instances; and yet we fail to learn those portents of disaster. The price of negligence is high – millions of clinical cases and tens of monies of infection prior to the emergence of the epidemic. Stool samples collected from China before April 2019 indicated SARS-CoV-2 genomic traces in 6 out of 26 samples (Rampelli et al., 2020). This testifies that transmission of the virus to humans had occurred before April 2019 fortifying the proposition that the virus was initially quite harmless, and that it had a great deal of time to undergo mutations. The ability of the virus to undergo rapid evolutionary changes is indubitable as genomic variations in different infected people showed. Population analysis revealed that the virus has been circulating in human in two distinct genomic types, named L and S. The S lineage is older and more benign with the prevalence rate of 30%; whereas the L type is the more virulent and more recent, with 70% prevalence (Tang et al., 2020). This is a further support to the human-only speciation hypothesis.

Conflict of Interest

The author declares that there is no conflict of interest.

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