

## Tracing the SARS-coronavirus

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### ABSTRACT

Four coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1) are endemic in humans and mainly associated with mild respiratory illnesses; whereas the other two coronaviruses [Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV)] present as emerging infections causing severe respiratory syndrome. Coronaviruses evolve by accumulation of point mutations and recombination of genomes among different strains or species. Mammalian coronaviruses including those infect humans are evolved from bat coronaviruses. While SARS-CoV and MERS-CoV are genetically closely related to bat coronaviruses, intermediate host(s) is (are) likely to be involved in the emergence and cross-species transmission of these novel human viruses. High prevalence of SARS-like coronaviruses have been found from masked palm civet cats and raccoon dogs collected from markets around the time of outbreaks in humans, but these animals are likely to be a transient accidental host rather than a persisting reservoir. More research is needed to elucidate the ecology of coronaviruses. Vigilance and surveillance should be maintained to promptly identify newly emerged coronaviruses.

### KEY WORDS

Severe acute respiratory syndrome (SARS); coronaviruses (CoV); evolution; animal reservoir

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### Coronavirus and human diseases

The family *Coronaviridae* comprises of a large group of positive-strand RNA viruses infecting diverse avian and mammalian species. Human coronavirus (HCoV) infections are typically mild, but with remarkable rare exceptions (1). Before 2003, only two coronaviruses (HCoV-229E and HCoV-OC43) were known to infect humans, and both produce mild self-limiting upper respiratory tract illnesses. In early 2003, outbreaks of severe acute respiratory syndrome (SARS) with unknown cause occurred in southern China, and then spread to other parts of the world (2,3). The aetiology was later proved to be infection with a novel coronavirus named as SARS-CoV which belongs to lineage B of the genus *Betacoronavirus* (4,5). The outbreak subsided by mid-2003, with subsequently a few laboratory-acquired cases and a small outbreak occurred in early 2004 (6,7). Altogether, there

were about 8,000 cases worldwide affecting almost 30 countries, with a case fatality rate of nearly 10% (8).

After 2003, there was a vigorously search for other possible human coronaviruses. In 2004, a previously unrecognized coronavirus was found, and named as HCoV-NL63 (9). The virus was discovered by non-target specific sequencing. HCoV-NL63 is genetically closely related to HCoV-229E, and utilizes angiotensin-converting enzyme 2 (ACE2) as a receptor (10). HCoV-NL63 produces mild respiratory illnesses. In 2005, another previously unrecognized coronavirus named HCoV-HKU1 was discovered (11). HCoV-HKU1 is distantly related to OC43, and the receptor has yet been identified. Generally, these HCoVs are associated with mild upper respiratory infection (12).

Recently, another novel coronavirus associated with severe acute respiratory syndrome has emerged in the Middle East, with the earliest known case dated back to April 2012 (13,14). This novel coronavirus virus, now named as Middle East Respiratory Syndrome Coronavirus (MERS-CoV), represents a new species in lineage C of the genus *Betacoronavirus*, which currently includes the *Tylonycteris* bat coronavirus HKU4 and *Pipistrellus* bat coronavirus HKU5 (5,15). By early June 2013, there were 55 laboratory-confirmed cases of MERS-CoV infections with 31 (56%) deaths and cases acquired the infection in Jordan, Kingdom of Saudi Arabia, Qatar and the United Arab Emirates (16). Of concern, family cluster of MERS-CoV infections and limited nosocomial transmission have been reported (17,18).

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Of the six coronaviruses known to infect humans, four (HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1) are endemic and cause, in general, mild upper respiratory illnesses. These coronaviruses have co-evolved with humans for a long period of time and thus adapted to survive with the human host. The other two coronaviruses (SARS-CoV and MERS-CoV) are definitely new to humans, and emerged as alarming outbreaks. While these novel "human" coronaviruses are believed to be originated from certain animal species, the path of transmission to humans remains a mystery.

### Evolution of coronavirus

With two novel viruses of the same family emerged within a period of 10 years, one would perceive coronavirus as a fast evolving virus. A pre-requisite of evolution is the ability to create diversity to allow selection of fitness. Being an RNA virus, coronaviruses rely on RNA-dependent RNA polymerase (RdRp) to replicate the virus genome. The intrinsic error rate of RdRp, in the order of magnitude of  $1 \times 10^{-6}$  mutation per site per replication (19,20), is a continuous source of point mutations for the replicating viral genome. Accumulation of these point mutations at critical sites might affect biological properties. As for comparison, the well-known stable virus family *Herpesviridae*, which relies on DNA polymerase to replicate its viral genome, has an error rate of about  $1 \times 10^{-8}$  (21). On the other hand, a typical example of high mutation rate is the family *Retroviridae*, which utilizes reverse transcriptase that has an error rate in the range of  $1 \times 10^{-4}$  to  $1 \times 10^{-5}$  (22). Thus, coronaviruses can be regarded as middle-ranking in terms of the ability to generate point mutations in viral genome during the process of virus replication.

Point mutation alone is not sufficient to create a novel virus such as SARS-CoV. Coronavirus can gain a genomic fragment of hundreds or thousands base-pair long from another coronavirus strain when they are co-infecting the same host. This process, known as recombination, is often more important than point mutations for viruses to expand or switch ecological niches (23). Coronaviruses are found in diverse species of animal (24). Recombination between these similar but different viruses can create novel strains with the ability to cross species barrier. While coronaviruses can acquire fragments of new genome through the process of recombination, their ability to acquire a big trunk of genome is not as good as influenza viruses that carry segmented genomes, which make the exchange of the whole segment of genome possible.

The wide distribution of coronaviruses in diverse avian and mammalian species poses a threat to humans. A novel virus with the ability to infect humans can be created by recombination among animal or avian virus strains. Furthermore, existing avian or mammalian virus strains can infect humans without

prior changes. Current phylogenetic analysis indicates that coronaviruses have evolved into two lineages (25). The avian lineage comprises gamma and delta coronaviruses infecting different avian species. The mammalian lineage comprises alpha and beta coronaviruses infecting bats, porcine, feline, murine, bovine, equine and humans. All coronaviruses circulating in humans are believed to be evolved from mammalian coronaviruses, namely the bat coronaviruses (24,25). It has been estimated that HCoV-OC43 was transmitted to humans from bats about 120 years ago, and HCoV-229E was transmitted from bats about 200 years ago; whereas the HCoV-NL63 was probably transmitted from bats about 500-800 years ago. Although the time of transmission of SARS-CoV and MERS-CoV to humans remains elusive, both viruses are, at least, phylogenetically linked to bat coronaviruses (26,27). The existence of a common ancestor of all known coronaviruses may be dated back to millions of years ago (28).

### Bats as an ancestor of emerging viruses

The fact that SARS-CoV might have emerged from bats did not come as a surprise. Before SARS, there was at least once in a decade a virus linked to bats had emerged to infect humans, including Marburg virus causing haemorrhagic fever in 1967, Ebola virus causing haemorrhagic fever in 1976, Hendra virus causing severe pneumonia in 1994, and Nipah virus causing neurological and respiratory diseases in 1998. Bats carry a lot of special features making them an ancestor or source of emerging viruses (29). It was originated about 50-52 million year ago with little changes till present time. It has more than 1,000 species and multiple species can exist in large colony sharing the same habit. This together large colony size of more than 200,000 and the long fly range of hundreds to thousands kilometers allow frequent recombination between virus strains. Most important is that bats often harbor multiple viruses for a long period and they are asymptomatic. These together with their long life span (3.5× longer than mammals of similar size) allow persistence and spreading of newly emerged virus strains.

### Bats and SARS-CoV

Coronaviruses have been detected from a number of bat species. The one most closely related to SARS-CoV was found in horseshoe bats (30). Among horseshoe bats, a high seroprevalence was found and anal swabs were positive by PCR. However, it is difficult to isolate the virus. These bat SARS-Like CoV shares 87-92% overall sequence homology with human and civet SARS-CoV, i.e., harbouring a 29-nt site considered to be a signature of civet and early human cases. The key difference between bat SARS-Like CoV and human SARS-CoV lies in the spike protein which is the receptor binding protein. The spike

protein has two domains designed as S1 and S2. S1 is responsible for attachment to the host cell-surface receptor ACE2. S2 is responsible for fusing with the host cell membrane. The S2 of bat SARS-Like CoV is about 96% identical to that of SARS-CoV suggesting both viruses share a similar mechanism of membrane fusion. In contrast, the sequence similarity between S1 proteins of bat SARS-Like CoV and SARS-CoV is low. While bat SARS-Like CoV is likely to be the ancestor of SARS-CoV, there is still an important genetic gap at S1 which needs to be filled up, probably, by recombination. So far, no SARS-CoV has been identified in bats and bats do not have the appropriate receptor (ACE2) for SARS-CoV (31). At present, little is known about where, when and in which animal species had this recombination occurred.

### Intermediate host and emergence of SARS-CoV

An intermediate host is likely to be involved in bridging the evolution gap between bat SARS-Like CoV and SARS-CoV. There are a few theoretical scenarios. Firstly, the intermediate host might be a “persistent” one that is still maintaining SARS-CoV infection within that species, and that species is quite remote from humans making transmission to humans a rare event. Secondly, the intermediate host species was only “transiently” infected for short period of time around 2002-2004, and it is no longer sustained in that species population. This could happen if that animal species only have a short period of infectivity, shedding a low level of viruses, low population density or high mortality rate following infection.

A number of studies have tried to detect SARS-CoV from animals. Masked palm civet cat is the most important one among all animal species that SARS-CoV have been found (32). The positive rate for SARS-CoV among civet cats was as high as 100% in markets at specific times; but was general negative among farms, except in rare occasions. Civet CoV has a 29-nt deletion that was found in some early human cases. Civet CoV single nucleotide variations (SNVs) were observed in early human cases, some SNVs remained in human cases infected at the middle of the epidemic, but none were found in human cases infected during the late phase. These observations together with the fact that the sequences among civet CoV isolates showed a high degree of homology suggesting that CoV was recently introduced into civet population and was still evolving. Most likely civet cats, like humans, were a transient accident host.

Raccoon dog is another species found to have high positive rate (100%) in live markets around the time of outbreaks in humans (33). The sequence of CoV detected from raccoon dog was identical to those from civet cats. However, relative little attention has been drawn to raccoon dog. Other animals in markets tested positive for CoV included domestic cat, red fox, Lesser rice field rat, goose, Chinese ferret-badger and wild boar (33). However, the

samples were collected at times when the markets were heavily contaminated. It is difficult to determine whether these animals were actively shedding or passively carrying CoV.

### The challenges ahead

SARS-CoV is only an example of emerging infections that we are going to face. In coming years, more cross-species infections are expected to happen. Factors influencing the chance of emergence of zoonotic infections are complex; and population density, ecology and proximity between animals and humans probably play a certain role. Close surveillance for emerging pathogens is necessary.

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