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## Editorial note:

Novel microorganisms are being discovered regularly, and among them are newly emerging pathogens. The SARS epidemic in 2003, eventually determined to be caused by a hitherto unknown coronavirus, has fuelled research in coronaviruses and interests in discovery of novel coronaviruses. In this article, Prof. Patrick Woo provided an overview on coronaviruses, and updates on recently discovered members, illustrating the complexity of the virology world, and that most probably a lot more still awaits to be discovered. We welcome any feedback or suggestions. Please direct them to Dr. Janice Lo (e-mail: janicelo@dh.gov.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

## Discovery of novel microbes: more and more coronaviruses after the SARS epidemic

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### Introduction

The *Coronaviridae* family is classified into two subfamilies, *Coronavirinae* and *Torovirinae*. Members of the *Coronavirinae* subfamily are in general referred to as coronaviruses. Phenotypically, coronaviruses are enveloped viruses of 120-160 nm in diameter. Under electron microscopy, coronaviruses have a crown-like appearance and the name “coronavirus” is derived from the Greek word *κορώνα*, which means crown. Genotypically, coronaviruses are positive-sense, single-stranded RNA viruses with genome sizes of about 30 kb, the largest genome size among all RNA viruses. Traditionally, coronaviruses were

classified into three groups based on their antigenic relationships. Groups 1 and 2 are made up of mammalian coronaviruses and group 3 avian coronaviruses. Recently, the Coronavirus Study Group of the International Committee for Taxonomy of Viruses (ICTV) has proposed three genera, *Alphacoronavirus*, *Betacoronavirus* and *Gammacoronavirus*, to replace these three traditional groups of coronaviruses. Before 2003, there were less than 10 coronaviruses with complete genomes available, with only two human coronaviruses, namely human coronavirus 229E (HCoV-229E) and human coronavirus OC43 (HCoV-OC43), which were discovered in the 1960s. The SARS epidemic in 2003 has

boosted interest in coronavirus research globally; and most notably, in the discovery of novel coronaviruses and their genomics. In the past six years, our group has discovered 13 novel coronaviruses, including one novel human coronavirus [human coronavirus HKU1 (HCoV-HKU1)], SARS-related *Rhinolophus* bat coronavirus (SARSr-Rh-BatCoV), eight other bat coronaviruses and three avian coronaviruses, and has sequenced the genomes of nine of them <sup>(1-5)</sup>. Others have also discovered additional coronaviruses, the most notable being human coronavirus NL63 (HCoV-NL63), discovered by a group in the Netherlands <sup>(6)</sup>.

### Human coronavirus HKU1

HCoV-HKU1 was discovered in 2005 from the nasopharyngeal aspirate of a 71-year old Chinese man with pneumonia <sup>(1)</sup>. Under the new classification system, HCoV-HKU1 belongs to *Betacoronavirus* subgroup A. Uniquely, its G + C content is 32%, the lowest among all known coronaviruses. Furthermore, it also shows the most extreme codon usage bias due to cytosine deamination <sup>(7)</sup>. Since its discovery, HCoV-HKU1 infections have been reported globally, with the highest incidence in the winter months <sup>(8-13)</sup>. It is associated with upper and lower respiratory tract infections. Most cases were self-limiting, with deaths only reported in two patients with multiple underlying diseases <sup>(8)</sup>. Recently, HCoV-HKU1 has been successfully cultivated using models of human ciliated airway epithelial cell culture <sup>(14)</sup>. By analyzing the genome sequences of 22 strains of HCoV-HKU1, three genotypes of HCoV-HKU1, named genotypes A, B and C, were observed <sup>(15)</sup>. These genotypes were generated as a result of multiple inter-genotypic homologous recombination events <sup>(15)</sup>. Laboratory diagnosis of HCoV-HKU1 infections was mainly achieved by amplifying the RNA-dependent RNA polymerase or nucleocapsid gene from nasopharyngeal aspirates using RT-PCR.

### SARS-related *Rhinolophus* bat coronavirus

Although SARS-related coronavirus (SARSr-CoV) was found in civets in live animal markets in mainland China during the SARS epidemic,

multiple lines of evidence suggested that civets were not the natural reservoir, but just the amplification hosts of SARSr-CoV. Therefore, in 2005, we carried out a territory-wide animal surveillance study in Hong Kong to look for the animal reservoir of SARSr-CoV. Results showed that a SARSr-CoV, named SARSr-Rh-BatCoV, was present in 39% of Chinese horseshoe bats (*Rhinolophus sinicus*) in Hong Kong, but not in other animals <sup>(2)</sup>. Others have also reported the presence of SARSr-Rh-BatCoV in other horseshoe bat species in other provinces of mainland China <sup>(16)</sup>. SARSr-Rh-BatCoV differed from SARSr-CoVs in humans in that the genomes of SARSr-Rh-BatCoV, but not those of most human SARSr-CoV genomes, contained a 29-bp insertion in ORF 8. This suggested that SARSr-Rh-BatCoV has a common ancestor with SARSr-CoV in civets. Together with SARSr-CoV in humans and civets, SARSr-Rh-BatCoV belongs to *Betacoronavirus* subgroup B. Recently, our tagging experiments in Chinese horseshoe bats and molecular clock analysis confirmed that SARSr-CoVs were newly emerged viruses and the time of the most recent common ancestor was in 1972, and the time of divergence for the civet and bat strains was in 1995 <sup>(17)</sup>.

### Other novel bat coronaviruses

The discovery of SARS-CoV in bats has led to a boost of interests in looking for more novel coronaviruses in bats. Among the eight additional bat coronaviruses we discovered, five [*Rhinolophus* bat coronavirus HKU2, *Myotis* bat coronavirus HKU6, *Miniopterus* bat coronavirus HKU7, *Miniopterus* bat coronavirus HKU8 and *Rousettus* bat coronavirus HKU10] belonged to *Alphacoronavirus* and three [*Tylonycteris* bat coronavirus HKU4 (Ty-BatCoV HKU4), *Pipistrellus* bat coronavirus HKU5 (Pi-BatCoV HKU5) and *Rousettus* bat coronavirus HKU9 (Ro-BatCoV HKU9)] belonged to *Betacoronavirus* <sup>(4,18)</sup>. Detailed phylogenetic analysis revealed that the three which belonged to *Betacoronavirus* constituted two novel subgroups, which were named subgroup C (Ty-BatCoV HKU4 and Pi-BatCoV HKU5) and subgroup D (Ro-BatCoV HKU9) respectively <sup>(4)</sup>. In general, bat coronaviruses are bat genus/species specific,

although one bat genus/species may be the reservoir of more than one coronavirus species. In 2009, the Coronavirus Study Group of the ICTV has unified the nomenclature of bat coronaviruses, using the format “genus of bat” (e.g. *Rhinolophus*) followed by “bat coronavirus” followed by “a unique part of the species of the virus” (e.g. HKU2), with the short form Rh-BatCoV HKU2. Recently, we have also discovered that more than one genotype of Ro-BatCoV HKU9 can co-exist in the same bat<sup>(19)</sup>.

### Novel avian coronaviruses

As birds are the reservoir of major emerging viruses but the number of known coronaviruses in birds is relatively small, we carried out a territory-wide coronavirus surveillance study in dead wild birds in Hong Kong<sup>(5)</sup>. In this study, three novel avian coronaviruses were discovered from three different families of birds (bulbuls, thrushes and munias) commonly found in Hong Kong<sup>(5)</sup>. These coronaviruses were named bulbul coronavirus HKU11 (BuCoV HKU11), thrush coronavirus HKU12 (ThCoV HKU12) and munia coronavirus HKU13 (MuCoV HKU13)<sup>(5)</sup>. Their genomes, with size ranged from 26.4 to 26.6 kb, represent the smallest known coronavirus genomes, despite the presence of the largest number of open reading frames downstream to the nucleocapsid gene. Phylogenetically, these three coronaviruses were distinct from the other known avian coronaviruses, such as the infectious bronchitis virus and its close relatives. Detailed phylogenetic analysis revealed that these three coronaviruses probably represented a novel genus, *Deltacoronavirus*, in the *Coronavirinae* subfamily.

### Concluding remarks

In the last few years, we have witnessed a tremendous boost in the number of novel coronaviruses discovered. With these, we are starting to appreciate more about coronavirus diversity and their hosts. Bat coronaviruses are believed to the gene pool of *Alphacoronavirus* and *Betacoronavirus* and bird coronaviruses the gene pool of *Gammacoronavirus* and *Deltacoronavirus*. The availability of sophisticated bioinformatics tools and a comprehensive and user-friendly

coronavirus database have also given us an unprecedented opportunity to learn more about coronavirus genomics and understand when and how interspecies jumping has occurred<sup>(20)</sup>.

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