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Coronaviruses, bats, and museums

The past two years of the COVID-19 pandemic has taught us a lot about emerging diseases, but also transmission of infections and ways of preventing future outbreaks from spreading globally. However, there is still much to be learned, such as how the culprit coronavirus SARS-CoV-2 mutates into different variants, what is the intermediate host that allowed the virus to jump to humans, and a better understanding of immune responses to this severe acute respiratory illness.

I am a museum curator with research interests in the evolution and biodiversity of mammals, including horseshoe bats in the genus *Rhinolophus* from Asia that have been implicated as a natural reservoir of coronaviruses. But they are asymptomatic and appear healthy so studying them will give us valuable insights into both the biology of the second most speciose group of mammals and the potential benefits to human medicine. A multidisciplinary approach is taken in this editorial to summarize pertinent details from research articles to give an integrative perspective on the relationships among coronaviruses, bats, and museums. This will be a different view than what would be found in traditional mainstream media stories and also dispel some myths that have appeared on social media. One of the more infamous posts suggested that the outbreak got started by people eating bat soup, but this turned out to be an old video on YouTube that pre-dated the pandemic and was not from China where the closest genetic SARS-like coronavirus is found (BBC Monitoring 2020). Unfortunately, this led to the ill-conceived killing of bats in several countries throughout the world (e.g. Goyal 2020).

There are four types or genera of coronaviruses: alpha and beta coronaviruses are found in mammals, whereas gamma and delta coronaviruses are found in birds. Within beta coronaviruses, they are separated into 4 subgenera, including Merbecovirus, Nobecovirus, Hibecovirus, and Sarbecovirus with the horseshoe bat family Rhinolophidae as the ancestral host for this virus genus (Latinne *et al*. 2020). More specifically, Sarbecoviruses occur in horseshoe bats and other mammals with two of these Sarbecoviruses each causing the SARS outbreak in 2003 and the current COVID-19 pandemic. Merbecovirus includes another beta coronavirus that caused the Middle East Respiratory Syndrome (MERS) virus or camel flu outbreak in 2012, which is a less contagious disease that was transmitted to humans from camels. Initially, similar viruses were detected in bats, making them suspected reservoirs, but they were found to be only distantly related to MERS (reviewed in Cheetham & Markotter 2021).

Bats have not been documented to transmit SARS-like sarbecoviruses directly to humans, which is spread through an unknown intermediate host, but there is a connection between bats and the highly contagious, deadly diseases linked to these viruses. The closest known virus to SARS-CoV-2 was found in the intermediate horseshoe bat (*Rhinolophus affinis*) from Yunnan, China, and was designated as RaTG13 (Zhou *et al*. 2020). However, there is a 4% difference in RNA between the two sarbecoviruses; as a comparison there is about a 5% difference in DNA between chimpanzees and humans. Using a coalescent analysis, the most recent common ancestor of the two sarbecoviruses was dated to 1969, which means that the virus has been circulating unnoticed for the past 5 decades in an unknown intermediate host before jumping to humans (Boni *et al*. 2020). Although the bat sarbecovirus RaTG13 is the most similar, it has 5 key amino acids that are different from SARS-CoV-2 in the important spike protein region that is involved in binding to the human ACE2 receptor site for gaining access to infect the host cell (Andersen *et al*. 2020). Therefore, bat sarbecoviruses probably are not readily infectious to humans. Pangolins were initially implicated as a possible intermediate host (Lam *et al*. 2020), however, they do not have an insertion of four amino acids at the spike protein polybasic cleavage site that is currently considered unique to the human coronavirus (Andersen *et al*. 2020). Hence, the transmission source to humans is still unknown and persecution of wildlife has been a dangerous consequence of premature conclusions (Frutos *et al*. 2020). Much of this initial

research has focused on sequencing the approximately 30 thousand nucleotides of the genomic RNA that comprise these sarbecoviruses to characterize their molecular structure, such as the spike protein receptor-binding domain and the polybasic cleavage site. However, more attention should now be given to sequencing the approximately 2 billion nucleotides of the genomic DNA of the putative natural reservoir host (horseshoe bats) to not only characterize their ACE2 receptor sites but also the immune system to tolerate or resist coronaviruses.

Bats are unique animals in several ways, including being the only flying mammals, with many adaptations in morphology and behaviour. Most species have a sophisticated form of laryngeal echolocation that helps them to navigate in total darkness and some also use it to find their food. Although there are a lot of myths associated with bats, such as Dracula and blood feeding, only 3 species of bats are sanguivorous and occur only in the Neotropics. Bats are also highly diverse with >1,450 species (Simmons & Cirranello 2022), which is the second most species-rich taxonomic order representing about 20% of mammalian diversity. In addition, they are beneficial to the environment and provide many ecosystem services such as seed dispersal, flower pollination, and insect predation, including eating agricultural pests that have economic impacts on farmers.

A consortium involving more than 150 researchers at universities and museums from around the world have formed the Bat1K project with the aim of generating chromosome-level genomes for all living species of bats (Teeling *et al*. 2018). Their objectives are separated into 3 phases: Phase 1 is to sequence a species of bat in each of the 21 families of bats, Phase 2 is a species in the 220 genera, and Phase 3 is getting the genomes of all 1,450+ species of bats (https://www.bat1k.com). Phase 1 is currently being completed and preliminary results from 6 species representing different families have revealed some of the fascinating evolutionary adaptations in bats for flying, echolocation, longevity, and immunity (Jebb *et al*. 2020). Phase 2 has begun to sequence the genomes of the different genera, including vampire bats that have molecularly adapted to blood feeding with the loss of many genes (Blumer *et al*. 2022). But the COVID-19 pandemic has also accelerated the need to start Phase 3 with targeted species coverage within the horseshoe bat genus *Rhinolophus*.

Museum collections have always been valuable repositories documenting biodiversity and evolution in both a spatial and temporal context. In addition to traditional voucher specimens of skins and skeletons, over the past few decades tissue samples have become more commonly preserved for molecular study of genetic variation. During the pandemic, the importance of these biobanks became even more apparent because fieldwork to collect new samples for coronavirus research was difficult with increased restrictions on international air travel and the concern with spreading the disease to humans, but also back to wildlife. Initially, tissue samples in natural history collections were typically preserved in ethanol for molecular analysis, such as protein electrophoresis, which was one of the more popular methods in the 1980s and earlier. With the advent of the polymerase chain reaction (PCR) technique for amplifying specific genes, Sanger sequencing of DNA became prevalent in genetic studies investigating biodiversity and evolution. However, newer third generation genomic sequencing technologies, including PacBio, require high molecular weight (HMW) DNA to recover long-read molecules approximately 150 kilobases (kb) in length for scaffolding of fragments during genome assembly (Yohe *et al*. 2019). Although more difficult from a logistical perspective, especially when conducting fieldwork in remote areas, flash freezing at -196°C in liquid nitrogen is the best method of preserving HMW DNA for whole genome sequencing. Likewise, liquid nitrogen is also necessary for preserving less stable RNA, even though these molecules are shorter, for transcriptomic studies of proteins or research on viruses such as SARS-CoV-2. For comparison, the older next generation and Sanger sequencing techniques can use other tissue types and less stringent preservation methods such as ethanol that yield low molecular weight (LMW) DNA of 20 kb, but these fragments are often too short for recovering good-quality chromosome-level genome assemblies or for RNAs.

Less than 30% of mammal collections in the Western Hemisphere have tissue samples associated with voucher specimens, although 90% of the 20 largest museums do have specimens with tissues (Dunnum *et al*. 2018). In addition, most tissue collections are probably preserved in ethanol so not ideal for genomic or transcriptomic sequencing. Therefore, there needs to be a change of standards to freeze more tissue samples in liquid nitrogen in the field with long-term storage in at least -80°C ultracold freezers to facilitate third generation sequencing methods. The Royal Ontario Museum (ROM) has routinely preserved tissue samples in liquid nitrogen since 1989, so is now a valuable

resource for the Bat1K initiative. Although there is good representation of diversity with 15 of 21 families of bats, 120 of 220 genera, and about 400 of 1,450 species of bats, more collecting will be needed to reach the goals of Bat1K. As a complement to sequencing bat genomes to find the molecular basis to their resistance or tolerance to SAR-CoV-2, the ROM frozen bat tissue collection is also being screened for potential coronaviruses. If found, the RNA will be extracted and the spike protein region will be targeted with the goal of developing a ready-made vaccine bank specific to the different viruses present to get a head start on future diseases (Oosthoek 2021). By using genomic approaches from both the bat host and the virus parasite perspectives, we hope to prevent the next coronavirus outbreak from becoming another global pandemic.

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Burton K. Lim

Department of Natural History
Royal Ontario Museum
Toronto, Ontario, M5S 2C6
Canada.

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