SURVEILLANCE FOR NOVEL CORONAVIRUSES IN WEST AFRICAN BATS

Makda Asrat[1], Isamara Navarrete-Macias[1], Annie Rein-Weston[1], Cassie Bonavita [1], Heather Wells[2], Simon J. Anthony[1]

[1] Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California Davis, Davis, CA

[2] Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, NY

BACKGROUND & RATIONALE

The first global pandemic of the 21st century was caused by SARS-Coronavirus (SARS-CoV-1). After emerging in southern China it rapidly spread to 27 countries, infected over 8,000 people and ultimately caused 774 known deaths. The current pandemic caused by SARS-CoV-2 has caused over 110 million confirmed cases of COVID-19 and over 2.4 million deaths (Johns Hopkins). In addition to loss of life, these outbreaks have caused substantial economic losses and have disproportionately impacted communities that are already marginalized and vulnerable (Daszak et al. 2021).

SARS-CoV-1 and SARS-CoV-2, like the majority of emerging infectious diseases, are zoonotic. Even taking into account recent increases in surveillance efforts, the rate of new pathogen emergence has been rising for several decades, and almost all known pandemics originated in animals (Jones et al. 2008; Morse et al. 2012). Furthermore, the most significant pandemics of the last 50 years have been caused by viral pathogens that originated in wildlife (Morse et al. 2012; Daszak et al. 2021), and several of these zoonotic viral pathogens have been linked back to bat species, including SARS-CoV-1 and -2 (Li et al. 2005; Zhou et al 2020). Coronaviridae has been established as a family of zoonotic concern, and there is accumulating evidence that the bat virome includes substantial viral diversity within this family (Anthony et al. 2017a; reviewed by Letko et al. 2020).

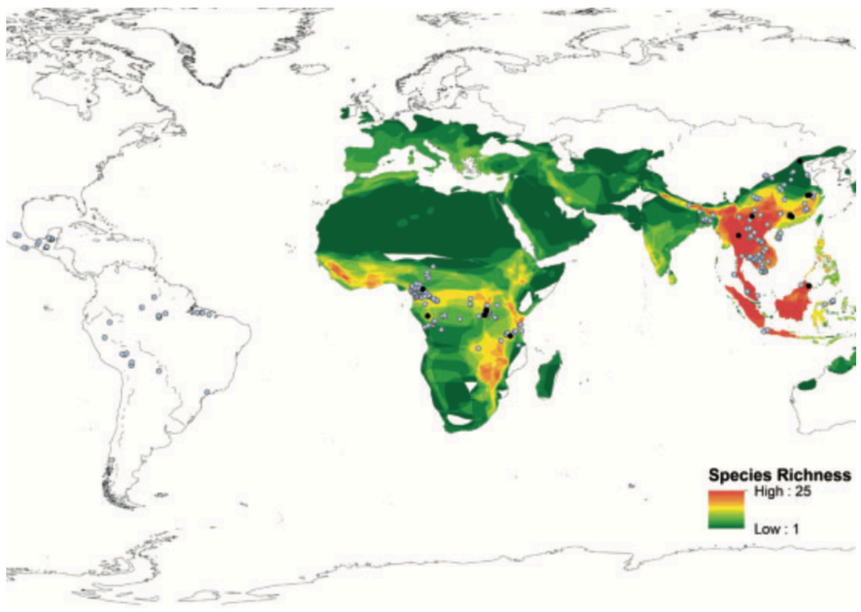


Figure 1: Map illustrating the potential distribution of sarbecoviruses based on the global distribution of bats in the *Rhinolophus* and *Hipposideros* genera. From Anthony et al., 2017.

Previous work has found that bats are the major wildlife reservoir of coronaviruses, including SARS-like coronaviruses. In addition, these studies found a significant association between particular lineages of CoVs and different bat species (Anthony et al. 2017a). SARS-like viruses (known as "sarbecoviruses") were found to be significantly associated with rhinolophid bats; thus, it follows that the distribution of sarbecoviruses is likely to mirror the distribution of rhinolophid bats.


Photo: iflscience.com

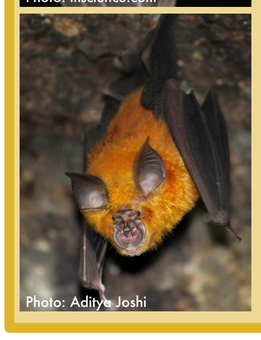


Photo: Aditya Joshi

Rhinolophid and hipposiderid bats are closely related sister genera. Rhinolophid bats are commonly known as horseshoe bats, in reference to the shape of their large nose-leaves. Hipposiderid bats are also called Old World leaf-nosed bats. Both genera echolocate by producing sound through their nostrils, and the large leaves on their noses aid in focusing echolocation.

Left: *Hipposideros ruber;*
Below & Right: *Rhinolophus landeri.*


Photo: Naskrecki & Guyton, naturepl.com


Photo: inaturalist.com

HYPOTHESIS

As West Africa has high species richness of rhinolophid bats, we hypothesize that there are undiscovered sarbecoviruses circulating in this region. This hypothesis is further supported by recent data showing the circulation of novel SARS-like viruses in Rwanda, where rhinolophid bats also circulate (Wells et al. 2021).

SPECIFIC AIMS

Aim #1: Survey bats in West Africa for novel coronaviruses using consensus polymerase chain reaction (cPCR). Clone and sequence all samples that test positive by cPCR using traditional Sanger sequencing.

Aim #2: Evaluate viral sequences using phylogenetics to determine their taxonomic placement.

METHODS

We used broadly reactive consensus polymerase chain reaction (cPCR) to identify known and novel coronaviruses in samples previously collected from bats in West Africa. The samples were collected as part of the Ebola Host Project (EHP) run by USAID PREDICT.

Consensus polymerase chain reaction (cPCR) is a broadly reactive PCR method that is inexpensive but highly effective for discovering novel viruses within known viral families or genera. cPCR uses degenerate primers — a mixture of closely related primer sequences with one or more positions that have several possible nucleotide bases — to bind and amplify parts of a viral genome that are conserved between all viruses within a given phylogenetic group. cPCR produces valuable population-level data on virus diversity, host-specificity and geographic range.

We used the Watanabe assay (Watanabe et al. 2010) to amplify a ~434 bp fragment of the RNA-dependent RNA polymerase (RdRp) within the orf1ab gene. Amplicons of the expected size were cloned and sequenced using traditional Sanger sequencing. Viral sequences were aligned in Geneious Prime v2021.2.2 (Biomatters Ltd., Auckland, NZ) using Clustal Omega v1.2.3 (Seivers, 2020). Viral sequences were then aligned to reference sequences gathered from GenBank using Mafft Multiple Alignment v1.4.0 (Biomatters Ltd., Auckland, NZ) to identify their taxonomic placement.

cPCR RESULTS & PHYLOGENETIC ANALYSIS

Total samples tested: **5696**

CoV positive results: **360**

- 161 *Hipposideros ruber*
- 191 *Miniopterus nimbae*
- 4 *Myonycteris angolensis*
- 4 *Rhinolophus sp.*

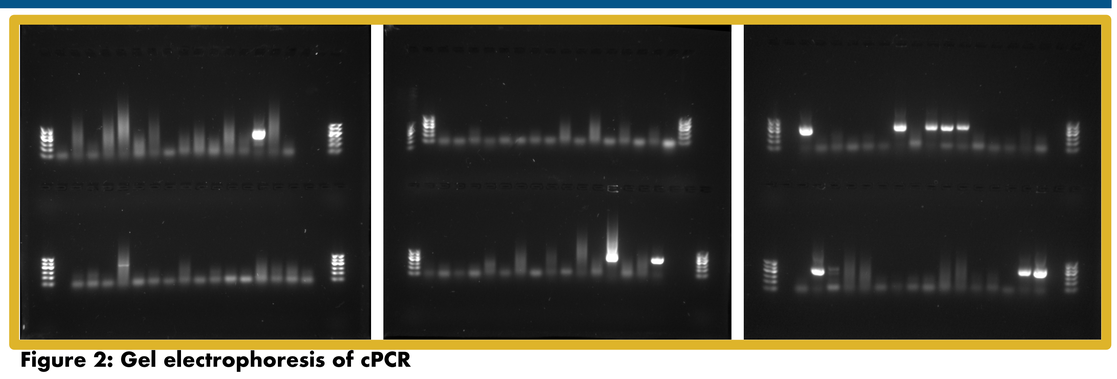


Figure 2: Gel electrophoresis of cPCR

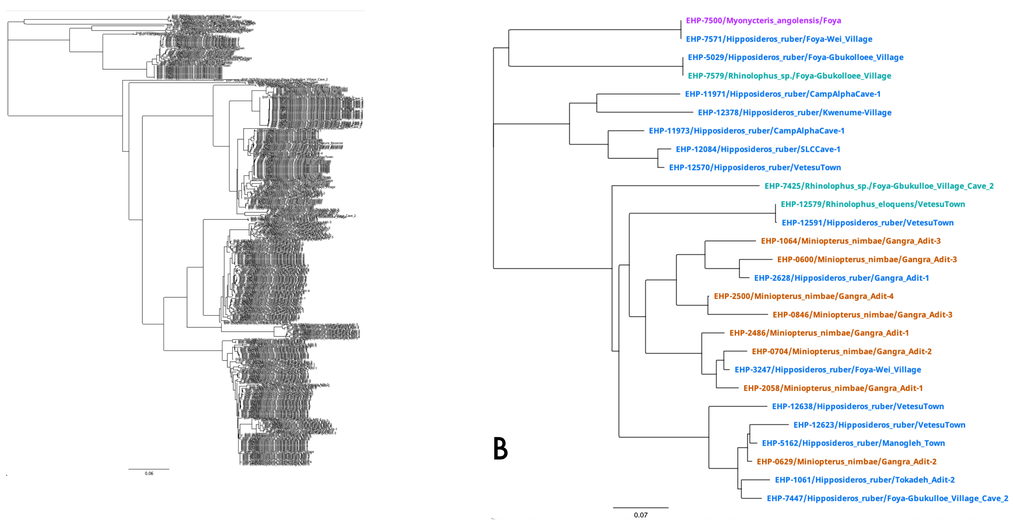
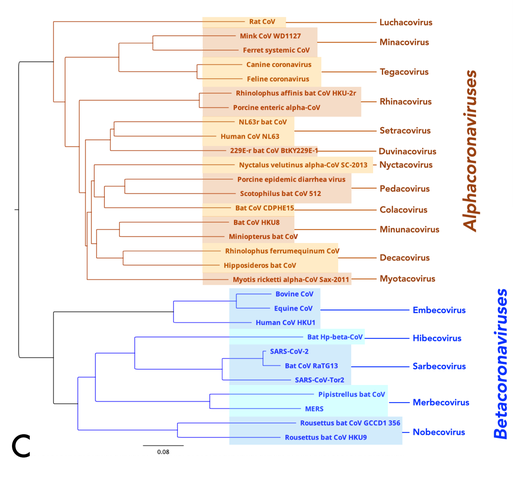


Figure 3: Phylogenetic analysis of CoV-positive cPCR products. Viral sequences were aligned in Geneious Prime v2021.2.2 (Biomatters Ltd., Auckland, NZ) using Clustal Omega v1.2.3 (Seivers, 2020). All trees were constructed in Geneious Prime using the neighbor-joining method. **A:** Phylogenetic tree showing taxonomic relationships between all 360 CoV-positive PCR amplicon sequences. **B:** A simplified version of tree [A] was made by selecting representative samples from each cluster of closely related viral sequences. The four different bat genera in which CoV-positive sequences were identified are shown in different colors. **C:** Tree [C] shows the phylogenetic relationships between known subgenera within the alpha- and betacoronavirus genera. Tree [C] was constructed using full genome sequences gathered from GenBank that were aligned and organized into a neighbor-joining tree in Geneious Prime. **D:** Tree [D] illustrates the relationship between trees [B] and [C], showing the representative samples of CoV-positive sequences aligned with existing, known sequences. **E:** Tree [E] is an enlargement of the betaCoVs from tree [D], with two sequences identified as most closely related to sarbecoviruses highlighted in lighter blue.



DISCUSSION

As illustrated in Fig. 3E, we identified two sequences that are most closely related to sarbecoviruses (highlighted in bright blue). We also found clusters of sequences that are most similar to hibecoviruses, which is noteworthy as that subgenus is most closely related to both merbeco and sarbecoviruses.

All of the viral sequences identified, in particular the betacoronaviruses highlighted above, are good candidates for full sequencing. Once contiguous genomes have been assembled they can be analyzed in downstream functional and evolutionary studies. We can verify the preliminary phylogenetic relationships found in this study, and further investigate their zoonotic potential.

In addition to identifying novel coronaviruses, the data generated by this study can also be used to help us better understand the natural history of these viruses and the forces driving their diversity, distribution and evolution.

ACKNOWLEDGMENTS

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