

RESEARCH NOTE

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Ecological surveillance of bat coronaviruses in Sarawak, Malaysian Borneo

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Abstract

Objective: Coronaviruses (CoVs) are natural commensals of bats. Two subgenera, namely Sarbecoviruses and Merbecoviruses have a high zoonotic potential and have been associated with three separate spillover events in the past 2 decades, making surveillance of bat-CoVs crucial for the prevention of the next epidemic. The study was aimed to elucidate the presence of coronavirus in fresh bat guano sampled from Wind Cave Nature Reserve (WCNR) in Sarawak, Malaysian Borneo. Samples collected were placed into viral transport medium, transported on ice within the collection day, and preserved at -80°C . Nucleic acid was extracted using the column method and screened using consensus PCR primers targeting the RNA-dependent RNA polymerase (RdRp) gene. Amplicons were sequenced bidirectionally using the Sanger method. Phylogenetic tree with maximum-likelihood bootstrap and Bayesian posterior probability were constructed.

Results: CoV-RNA was detected in ten specimens (47.6%, $n = 21$). Six alphacoronavirus and four betacoronaviruses were identified. The bat-CoVs can be phylogenetically grouped into four novel clades which are closely related to Decacovirus-1 and Decacovirus-2, Sarbecovirus, and an unclassified CoV. CoVs lineages unique to the Island of Borneo were discovered in Sarawak, Malaysia, with one of them closely related to Sarbecovirus. All of them are distant from currently known human coronaviruses.

Keywords: Bat, Cave, Coronavirus, Guano, Sarawak

Introduction

Coronaviridae consists of a large family of viruses that cause infections and diseases in a large range of vertebrates. Coronaviruses (CoVs) can be classified into four genera, *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. Both *Alphacoronavirus* and *Betacoronavirus* houses CoVs of public health importance. Currently, there are seven known human CoV types, 2002/03 Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), 2012 Middle Eastern Respiratory Syndrome CoV (MERS-CoV), 2019 SARS-CoV-2, and four seasonal CoVs (HCoV-229E, HCoV-NL63,

HCoV-OC43 and HCoV-HKU1) in which nearly all of them can be traced to their bat origin [1, 2]. The discovery of two bats CoVs, KSA-287 from *Taphozous perforatus* and RaTG13 from *Rhinolophus affinis* with 100% and 96% sequence identity to MERS-CoV and SARS-CoV-2 respectively, sealed the hypothesis that these CoVs of Public Health Emergency of International Importance (PHEIC) indeed have originated from bats. However, the progenitor for SARS-CoV was never discovered.

The SARS-CoV epidemic started in Guangdong, China in 2002, rampaging through numerous countries for nine months, infecting 8096, claiming the lives of 774. A decade later, MERS-CoV emerged in the Kingdom of Saudi Arabia, but cases have mostly been reported around the Middle East, Africa and South Asia with occasional exported cases [3]. As of June 2021, there were 2564 confirmed cases of MERS, including 886 associated death,

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