Identification of Coronaviruses in Bat Excreta in a Selected Semi-Urban Area in the Kandy District of Sri Lanka

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Bats are known asymptomatic reservoirs of many coronaviruses (CoV). Their dense roosting behavior promotes transmission and evolution of these viruses, posing zoonotic risks. Identifying CoV in bat colonies will help recognize emerging CoV, particularly in areas where human-animal interactions are common. This study aims to detect the presence of CoV in bat guano collected from a semi-urban bat colony in a selected site in the Kandy District of Sri Lanka. A total of 50 fresh bat guano were collected from a bat colony from Akurana, Sri Lanka in July 2023. Each guano was mixed separately with 1mL of Phosphate Buffered Saline (PBS). Viral RNA was extracted using Bioflux Biospin Virus DNA/RNA extraction kit, as per manufacturer's instructions. RNA extracts were subjected to a conventional PanCoV nested RT-PCR, which is designed to target RNA-dependent RNA polymerase gene with a 442 base pair fragment, a conserved segment in the *Orthocoronavirinae* family. Subsequently, PCR products were visualized using gel electrophoresis using a UV transilluminator. Finally, DNA sequencing was done for the positive samples using Oxford Nanopore Technology. Of the 50 bat guano samples tested, 15 (30%) were confirmed positive for CoV through PanCoV RT-PCR. Of the 15 CoV positive samples, 13 were successfully sequenced; the majority of these samples were classified as beta CoV using the NCBI blast. Identification of beta CoV in 30% of bat guanos in a semi-urban area of the Kandy District, emphasizes the need for enhanced surveillance of bats, especially in underrepresented areas, to understand CoV diversity to mitigate potential zoonotic spillovers that could pose threats to the public.

Keywords: Coronaviruses, bats, zoonotic, spillover, Sri Lanka