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Detection of coronaviruses in *Pteropus* & *Rousettus* species of bats from different States of India

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***Background & objectives*: Bats are considered to be the natural reservoir for many viruses, of which some are potential human pathogens. In India, an association of *Pteropus medius* bats with the Nipah virus was reported in the past. It is suspected that the recently emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) also has its association with bats. To assess the presence of CoVs in bats, we performed identification and characterization of bat CoV (BtCoV) in *P. medius* and *Rousettus* species from representative States in India, collected during 2018 and 2019.**

***Methods*: Representative rectal swab (RS) and throat swab specimens of *Pteropus* and *Rousettus* spp. bats were screened for CoVs using a pan-CoV reverse transcription-polymerase chain reaction (RT-PCR) targeting the RNA-dependent RNA polymerase (*RdRp*) gene. A single-step RT-PCR was performed on the RNA extracted from the bat specimens. Next-generation sequencing (NGS) was performed on a few representative bat specimens that were tested positive. Phylogenetic analysis was carried out on the partial sequences of *RdRp* gene sequences retrieved from both the bat species and complete viral genomes recovered from *Rousettus* spp.**

***Results*: Bat samples from the seven States were screened, and the RS specimens of eight *Rousettus* spp. and 21 *Pteropus* spp. were found positive for CoV *RdRp* gene. Among these, by Sanger sequencing, partial *RdRp* sequences could be retrieved from three *Rousettus* and eight *Pteropus* bat specimens. Phylogenetic analysis of the partial *RdRp* region demonstrated distinct subclustering of the BtCoV sequences retrieved from these *Rousettus* and *Pteropus* spp. bats. NGS led to the recovery of four sequences covering approximately 94.3 per cent of the whole genome of the BtCoVs from *Rousettus* bats. Three BtCoV sequences had 93.69 per cent identity to CoV BtRt-BetaCoV/GX2018. The fourth BtCoV sequence was 96.8 per cent identical to BtCoV HKU9-1.**

***Interpretation & conclusions*: This study was a step towards understanding the CoV circulation in Indian bats. Detection of potentially pathogenic CoVs in Indian bats stresses the need for enhanced screening for novel viruses in them. One Health approach with collaborative activities by the animal health and human health sectors in these surveillance activities shall be of use to public health. This would help in**

the development of diagnostic assays for novel viruses with outbreak potential and be useful in disease interventions. Proactive surveillance remains crucial for identifying the emerging novel viruses with epidemic potential and measures for risk mitigation.

Key words Bats - coronavirus - India - next-generation sequencing - phylogenetic - reverse transcription-polymerase chain reaction

A large number of emerging infectious diseases are known to be zoonotic in origin. In the last two decades, many viruses have been identified from bat species[1]. Bats have been recognized as the natural reservoirs of a variety of pathogenic viruses such as Rabies, Hendra, Marburg, Nipah and Ebola virus[2]. Bats are known to harbour coronaviruses (CoVs) and serve as their reservoirs. Alpha-CoV (α-CoV) and beta-CoV (β-CoV) have been detected in bats in Asia, Europe, Africa, North and South America and Australasia[3]. In the last two decades, bat CoVs (BtCoVs) garnered considerable attention as potential human pathogens[4,5]. Severe acute respiratory syndrome (SARS)-CoV-2 causing the current pandemic [CoV disease 2019 (COVID-19)] is also a member of the same genus and found to be similar to bat-derived CoV strain RATG13[6]. SARS-CoV-2 is reported to be 96 per cent identical to BtCoV at the whole genome level, and related viruses were identified in the previously sampled bat population in China[7].

CoVs are enveloped, single-stranded, positive-sense RNA viruses with a comparatively large genome size of 26 to 32 kb, classified under the family *Coronaviridae* in the order Nidovirales[8]. According to the International Committee on Taxonomy of Viruses (ICTV), they are classified into four genera, namely, α-CoV, β-CoV, γ-CoV and δ-CoV)[9]. β-CoVs are further classified into four different lineages [lineage A (L_A), lineage B (L_B), lineage C (L_C) and lineage D (L_D)][10]. Most of the human CoVs are either zoonotic in origin or circulate in animals[11]. CoVs can cause a wide range of infections, including respiratory tract infections, gastroenteritis, hepatitis and encephalomyelitis in their respective hosts. It is believed that many of the currently circulating α-CoVs and β-CoVs of mammals have evolutionary links to CoVs from bats[1].

India has a diverse population of bats; around 117 species of bats have been recorded, with around 100 subspecies coming under 39 genera belonging to eight families (*Pteropodidae*, *Rhinolophidae*, *Hipposideridae*, *Megadermatidae*, *Rhinopomatidae*, *Emballonuridae*, *Molossidae* and *Vespertilionidae*)[12]. The Indian Council of Medical Research-National Institute of Virology (ICMR-NIV) at Pune, India, has detected several viruses in bats, including the Nipah virus in *Pteropus medius*, Malsoor virus, Tioman virus and a novel adenovirus in *Rousettus leschenaultii*[13-15]. Nipah viral RNA antibodies could be detected in *Pteropus* bats from many States of India, and the possible link of transmission from bats could be established during the Nipah outbreak which occurred in Kerala in 2018 and 2019[16,17]. The use of conventional polymerase chain reaction/reverse transcription-polymerase chain reaction (PCR/RT-PCR), as well as metagenomics and next-generation sequencing (NGS) technologies, has led to the discovery of many novel viruses in bats. The identification of new CoVs in bats in several neighbouring Asian countries such as China[3], Sri Lanka[18] and Singapore[19,20] and the growing threats of novel CoV diseases such as COVID-19 led us to investigate *Pteropus* and *Rousettus* bats commonly found in India, for identification and characterization of BtCoVs.

Material & Methods

This study was approved by the Institutional Animal Ethics Committee (IAEC) of ICMR-NIV, Pune (IAEC/2019/MEZ/04). Permissions were also obtained from the Principal Chief Conservators of Forests (PCCF)/wildlife wardens of different States/ Union Territories (UT) (Kerala, Karnataka, Tamil Nadu, Himachal Pradesh, Punjab, Gujarat, Odisha, Telangana, Chandigarh and Puducherry).

Study sites and sample collection: Upon obtaining permission from the respective State authorities, bat-roosting sites in each State/UT were identified with the help of the forest officials. Bats were trapped using mist nets and were chemically restrained using isoflurane anaesthesia. Throat swabs (TS) and rectal swabs (RSs) were collected in virus transport medium (VTM) and were transported to ICMR-NIV, Pune, on dry ice. The specimens were collected from *Pteropus* spp. bats from

Kerala, Karnataka, Chandigarh, Gujarat, Himachal Pradesh, Odisha, Puducherry, Punjab, Tamil Nadu and Telangana and *Rousettus* spp. bats from Kerala, Karnataka, Chandigarh, Gujarat, Odisha, Punjab and Telangana States during 2018-2019. These bats were monitored and released after recovery. Twelve bats that died during the trapping process were transported to ICMR-NIV on dry ice. Necropsy of these bats was carried out in the Biosafety Level 4 (BSL-4) containment facility, and tissue specimens (intestine and kidney) collected were tested.

Detection of bat coronavirus using RT-PCR: RNA was extracted from the bat specimens using the MagMAX pathogen RNA/DNA isolation kit (Invitrogen, USA). RT-PCR was performed using Superscript III one-step RT-PCR (Invitrogen, USA) with Platinum High-Fidelity *Taq* polymerase (Invitrogen, USA) using the published BtCoV-specific primers targeting the conserved region of RNA-dependent RNA polymerase (*RdRp*) gene[21]. The amplicon of 440 bp was separated on 1.5 per cent agarose gel and visualized under VersaDoc MP 4000 ultraviolet transilluminator (Bio-Rad, USA).

Sequencing of the positive coronavirus specimens

Sanger sequencing of bat coronavirus: The RT-PCR products were separated on 1.5 per cent agarose gel, and 440 bp bands were excised. The excised gels were extracted and purified using a QIAQuick gel extraction kit (Qiagen, Hilden, Germany). The purified products were quantified, and chain-terminated PCR reactions were performed using pathogen-specific forward and reverse primers[21] with the BigDye Terminator 3.1 sequencing kit (Applied Biosystems, USA). BigDye reactions were purified using the DyeEx 2.0 spin kit (Qiagen, Germany). The purified chain-terminated reactions were sequenced using the ABI PRISM® 3100 Automated DNA Sequencer (Thermo Fisher Scientific, USA). The sequence data generated were assembled using the Sequencer 5.1 software (Accelrys Inc., USA).

Next-generation sequencing (NGS) of bat coronavirus: Selected bat specimens were used for RNA extraction[22,23]. RNA libraries were prepared and quantified by Qubit® 2.0 Fluorometer (Invitrogen, USA). NEB NextrRNA depletion kit (New England Biolabs, USA) was used to remove host ribosomal RNA and re-quantified using Qubit® 2.0 Fluorometer (Invitrogen, USA). In brief, the RNA library preparation involved fragmentation, adenylation, adapter ligation and amplification. The amplified libraries were quantified using KAPA Library Quantification Kit (KapaBiosystems, Roche, Switzerland) as per the manufacturer's protocol and further loaded onto the Illumina Miniseq NGS platform (Illumina, USA)[22,23].

The FASTQ files generated after the completion of the run were analyzed using CLC Genomics Workbench software version 11 (CLC, Qiagen, Germany). *De novo* assembly programme was used to assemble contiguous sequences (contigs). The contigs generated were analyzed using BLAST (*https://blast.ncbi.nlm.nih.gov/Blast.cgi*) to identify matching sequences. The closest matching sequence from GenBank (*https://www.ncbi.nlm.nih.gov/genbank/*) was used for reference mapping.

Phylogenetic analysis of partial and complete genome sequences of bat coronavirus: The CoV sequences retrieved from RS specimens of *Rousettus* spp. bats (n=4) were aligned with whole-genome sequences from GenBank using the create alignment function of the CLC genomics workbench (*https://digitalinsights.qiagen.com*). Partial *RdRp* gene sequences (~419 bp) retrieved by Sanger sequencing, for both the bat species specimens (genomic location: 14,701-15,120) were used to construct a phylogenetic tree along with the available *RdRp* sequences in GenBank. Phylogenetic analysis was carried out using the neighbour-joining method available in MEGA v7 software[24] using the Kimura 2-parameter nucleotide (nt) substitution model with 1000 bootstrap replicates. The nt divergence for the open reading frame (*ORF*) 1a polyprotein (*ORF* 1a), *ORF* 1b polyprotein (*ORF* 1b), spike protein (*S*), nucleocapsid phospoprotein (*N*), envelope protein (*E*) and membrane glycoprotein (*M*) genes was estimated using the Kimura 2-parameter model as implemented in the MEGA software. The sequences retrieved in the current study, along with those downloaded from GenBank, were grouped into the genus.

The viruses from the β-CoV genus were further grouped into lineages, L_A, L_B, L_C and L_D, to estimate the evolutionary divergence over the respective gene sequence pairs between groups using the MEGA software[24]. The distance was estimated using a Kimura 2-parameter model with uniform rates among the sites. The bootstrap of 500 replicates was used to estimate the variation in the model.

Results

The TS and RS specimens for 78 *Rousettus* spp. bats were collected in VTM from seven States (Kerala, Karnataka, Chandigarh, Gujarat, Odisha, Punjab and Telangana). The TS and RS specimens of 508 *Pteropus* spp. bats were also collected in VTM from 10 States/UTs in India (Kerala, Karnataka, Chandigarh, Gujarat, Himachal Pradesh, Odisha, Puducherry, Punjab, Tamil Nadu and Telangana). During the trapping process, 12 (8 *Rousettus* and 4 *Pteropus* spp.) bats died. Organ specimens (intestine and kidney) were collected from these bats (TS and RS specimens of these 12 bats were included in the total number of samples).

Detection of bat coronavirus using RdRp gene RT-PCR: Four of the 78 RS of *Rousettus* spp. bats screened for the BtCoV were found positive. All the positive RS samples belonged to Kerala State. Intestinal specimens of two bats were also found to be positive for the BtCoV. One bat (MCL-19-Bat-606), from Kerala, was tested positive in both the intestinal specimen and the RS. The second bat (MCL-20-Bat-76), from Karnataka, was tested positive only in the intestinal specimen. Altogether, five *Rousettus* spp. bats were positive for the BtCoV. All TS specimens from *Rousettus* spp. were found negative for BtCoV (Table I).

Twenty one of the 508 RSs from *Pteropus* spp. bats screened were tested positive for the BtCoV (Table I). These positive bats belonged to Kerala (n=12), Himachal Pradesh (n=2), Puducherry (n=6) and Tamil Nadu (n=1). The TS specimens of the same bats were tested negative for BtCoV. The TS specimens of RS-negative (n=42) bats were also screened and found to be negative (Table I). A total of 25 bats from both the species were found positive.

Sequencing of the positive coronavirus specimens

Sanger sequencing of bat coronavirus: Using the Sanger sequencing protocol, partial *RdRp* sequences of BtCoV were retrieved from two (out of 4 amplicons) specimens of *Rousettus* spp. One of the sequences (MCL-19-bat-588/2) showed close identity to BtCoV HKU9-5-2 (AN): HM211099.1; sequence identity (SI): 99.2 per cent, whereas the second *RdRp* sequence (MCL-20-bat-76/10) had an SI of 98.8 per cent with BtCoV HKU9-1 (AN: EF065513.1), both from China.

Sanger's sequencing protocol led to retrieval of eight partial *RdRp* sequences which belonged to *Pteropus* spp. These bats were collected from Kerala (n=5) and Tamil Nadu (n=3) States. One of the three partial *RdRp* sequences from Tamil Nadu had 97.93 per cent SI with BtCoV/B55951/Pte_lyl/CB2-THA (AN: MG256459.1, Thailand). The other two sequences had a minimum of 99.48 per cent SI with the CoV PREDICT_CoV-17/PB072 (AN: KX284942.1, Nepal). One of the five partial *RdRp* sequences from Kerala had 98.88 per cent SI with BtCoV/B55951/Pte_lyl/CB2-THA (AN: MG256459.1, Thailand). The remaining

Table I. Bat coronavirus positivity in bat specimens screened using RNA-dependent RNA polymerase (*RdRp*) gene-specific reverse transcription-polymerase chain reaction (RT-PCR) in different States

Place of collection	Number of positive/number tested (%) for different bat species for BtCoV *RdRp* gene-specific RT-PCR			
	Pteropus bats (%)		*Rousettus* bats (%)	
	Rectal swabs	Throat swabs	Rectal swabs	Throat swabs
Kerala	12/217 (5.53)	0/21 (0.00)	4/42 (9.52)	0/4 (0.00)
Karnataka	0/78 (0.00)	NT	0/4 (0.00)	0/4 (0.00)
Chandigarh	0/27 (0.00)	NT	0/6 (0.00)	0/6 (0.00)
Gujarat	0/30 (0.00)	NT	0/18 (0.00)	0/18 (0.00)
Odisha	0/30 (0.00)	NT	0/2 (0.00)	0/2 (0.00)
Punjab	0/14 (0.00)	NT	0/2 (0.00)	0/2 (0.00)
Telangana	0/30 (0.00)	NT	0/4 (0.00)	0/4 (0.00)
Himachal Pradesh	2/29 (6.89)	0/6 (0.00)	NA	NA
Puducherry	6/23 (26.09)	0/10 (0.00)	NA	NA
Tamil Nadu	1/30 (3.33)	0/5 (0.00)	NA	NA
	21/508 (4.13)	0/42 (0.00)	4/78 (5.13)	0/40 (0.00)

NT, not tested; NA, not available; BtCoV, bat coronavirus

Table II. Details of the genome recovered reads mapped and the per cent of reads mapped from the *Rousettus* bat samples

Sample details	Sample type	Virus retrieved	Relevant reads	Per cent of reads	Per cent of genome recovered
MCL-20-Bat-76	Kidney	Coronavirus BtRt-BetaCoV/GX2018	1632	0.015	94.39
	Intestine	BtCoV HKU9-1	4499	0.056	95.75
MCL-19-Bat-606	Rectal swab	Coronavirus BtRt-BetaCoV/GX2018	13,973	0.114	99.53
	Intestine	Coronavirus BtRt-BetaCoV/GX2018	10,214,492	93.476	99.87

four partial *RdRp* sequences had >97 per cent SI with CoV PREDICT_CoV-17/PB072 (AN: KX284942.1, Nepal).

Next-generation sequencing of bat coronavirus: NGS was performed on 10 specimens [4 RS, 2 kidney and 4 intestinal tissue) of the five *Rousettus* bats to retrieve the complete genome of the BtCoV. Kidney and intestine tissues of the bats from Karnataka State (MCL-20-Bat-76) and RS along with intestine tissue of bats from Kerala State (MCL-19-Bat-606) were used for sequencing and analysis.

Two different viruses were retrieved based on the BLAST analysis of the sequences from the kidney and intestine tissues of the bats from Karnataka. Kidney specimen of MCL-20-Bat-76 had an SI of 94 per cent and query coverage (QC) of 94 per cent with CoV BtRt-BetaCoV/GX2018 (AN: MK211379.1), whereas the intestine tissue of the MCL-20-Bat-76 had an SI of 96.8 and 95 per cent QC with the BtCoV HKU9-1 (AN: EF065513.1). The sequences from RS and intestine tissue of the MCL-19-Bat-606 from Kerala, had 93.69 and 93.99 per cent SI to CoV BtRt-BetaCoV/GX2018 (AN: MK211379.1), respectively, with 100 per cent QC. Further, 99.8 per cent of the CoV BtRt-BetaCoV/GX2018 sequences were retrieved from the intestine specimen of the MCL-19-Bat-606. The details of the genome recovered reads mapped and the per cent of reads mapped are summarized in Table II.

Phylogenetic analysis of partial and complete genome sequences of bat coronavirus: A neighbour-joining tree was generated using the partial *RdRp* region sequences derived from *Pteropus* and *Rousettus* spp. bat specimens. It was observed that all the BtCoV sequences were clustered within the L_D sequences of beta CoVs. A distinct subclustering of the sequences retrieved from *Pteropus* and *Rousettus* spp. bats is shown in Figure 1. The sequences in the light pink colour are retrieved from the *Pteropus* spp., whereas those in the dark pink region belong to *Rousettus* spp. The sequence divergence of 0.35 was observed between *Pteropus* spp. and *Rousettus* spp., which was obtained by averaging over all the sequence pairs between the two species, determining those to be distinct sequences to each species.

The complete genome sequences of four BtCoV obtained from *Rousettus* spp. specimens were used for generating a neighbour-joining tree (Fig. 2). These sequences were also clustered within L_D of β-CoVs as observed for partial *RdRp* sequence tree. These complete genome sequences were grouped into gene pairs to identify the gene with higher and lower divergence. The complete genomes of the Indian BtCoV sequences were grouped under L_D. The evolutionary divergence of *ORF* 1b was <0.54 between the different β-CoV lineages with a maximum score of 0.7 between different BtCoV sequences used in this study (Table III). *E* gene sequences had larger divergence within the β-CoV genus ranging from 2.18 to 0.94. Lineages L_A and L_C had the maximum divergence of 2.18, whereas the L_B and L_C were the least (0.94). *N* gene has an overall higher divergence among different lineages (ranging: 2.08-0.75). Overall, evolutionary divergence for the sequences of each gene pair demonstrated that *S*, *N*, *E* and *M* genes from the α- and δ-CoV highly diverged across the different genus. In contrast, the *ORF* 1b was less divergent across the genera (Table III).

Discussion

As per the available information, the BtCoV causing human infection belongs to α- and β-CoV genera of the *Coronaviridae* family. β-CoV genus has five strains known to infect humans[25]. The two human-infecting strains (NL63 and 229E) from α-CoV genus which cause mild-to-moderate respiratory infections are believed to have originated in bats[25]. Two members of the β-CoV genus (HCoV-OC43 and HCoV-HKU1) are known to cause the common cold and lower respiratory tract infections[26]. The other three

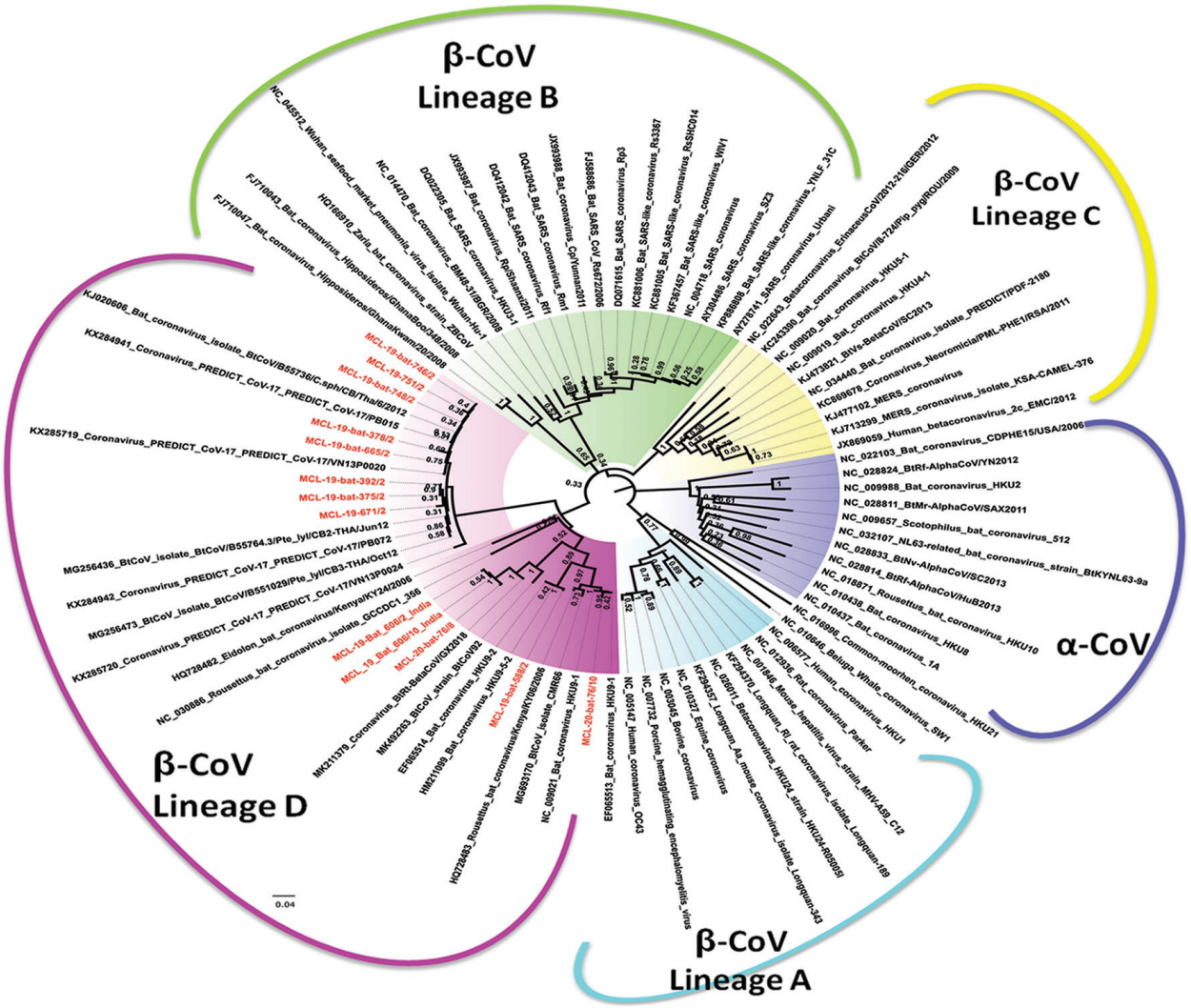

Fig. 1. Neighbour-joining tree for the RNA-dependent RNA polymerase (*RdRp*) partial sequence (genomic location: 14,701-15,120) generated from Sanger sequencing. The tree was constructed using the *RdRp* sequence available in the GenBank sequences. Kimura 2-parameter model was used as the substitution model to generate the tree. A bootstrap replication of 1000 cycles was performed to generate the tree to assess the statistical robustness.

are now shown to be pathogenic to humans (SARS-CoV-1, MERS-CoV and SARS-CoV-2). The SARS-CoV-1 and SARS-CoV-2 belong to L_B and MERS CoV belongs to L_C of β-CoV genus[27].

The phylogenetic analysis for the partial *RdRp* region revealed the presence of distinct BtCoVs in both the bats. The genomic sequences retrieved from the Indian sequences form a distinct cluster. The three CoV_BtRtBetaCoV/GX2018 sequences retrieved from the Indian *Rousettus* bats were 5.8-6.7 per cent different from the reference sequence, which was retrieved from *Rhinolophus affinis*. The two CoV_BtRtBetaCoV/GX2018 sequences retrieved from different bats were 1.2 per cent different from each other. The effect of host influence on the nt usage of the virus cannot be denied; however, it needs to be explored further in detail.

Bats are reservoirs for viruses with human pathogenic potential[28,29], and are known to harbour a broad range of CoVs[1]. The global distribution of bats, along with the different types of cell receptors present within them, favours virus replication, and is a possible link to their intraspecies transmission. The interspecies spill-over of a BtCoV to humans

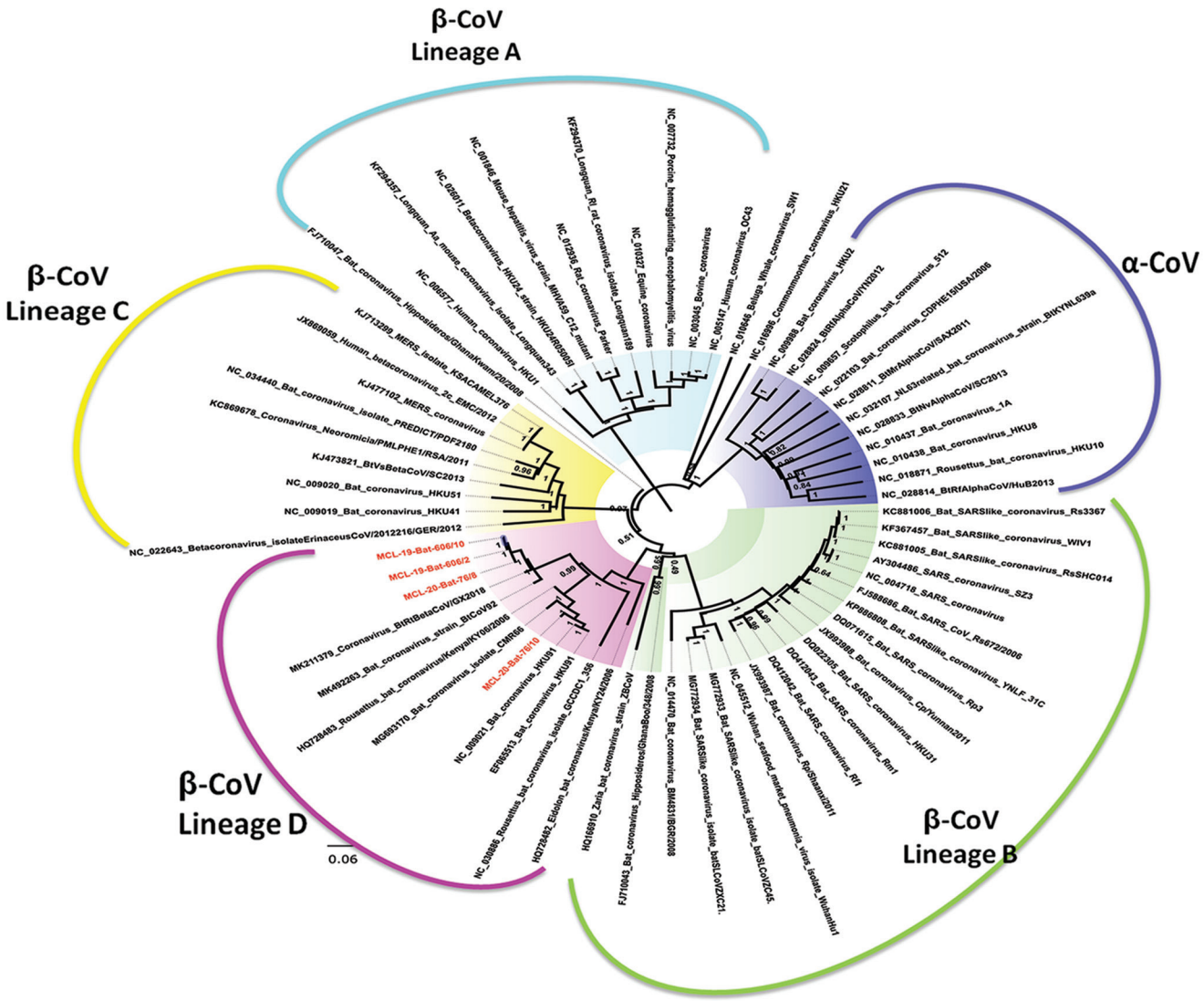

Fig. 2. Phylogenetic tree for the complete genome tree: A neighbour-joining tree was generated using the representative complete genome sequence available in the GenBank sequences. Kimura 2-parameter model was used as the substitution model to generate the tree. A bootstrap replication of 1000 cycles was performed to generate the tree to assess the statistical robustness.

is thought to occur through an intermediate host, in which the virus replicates through yet completely unidentified routes. In India, regions of the Western Ghats, particularly in Kerala, are reported to have habitat for diverse bat populations. The reports of pathogenic human viruses from bat specimens demand enhanced methods to monitor human exposure to various bat species. Investigations in unexplored regions/States should be focused on gaining further insights into CoV diversity within Indian bat populations.

Earlier, we had reported the presence of pathogenic viruses such as the Nipah virus in *Pteropus* bats in India[16]. In the present scenario of changing demography and ecological manipulations, it is challenging to have checks on the encounters of bats with other animals and humans. Therefore, active and continuous surveillance remains crucial for outbreak alerts for bat-associated viral agents with epidemic potential, which would be helpful in timely interventions.

Although CoVs in the subfamily *Coronavirinae* do not usually produce clinical symptoms in their natural hosts (bats), accidental transmission of these viruses to humans and other animals may result in respiratory, enteric, hepatic or neurologic diseases of variable

Table III. Evolutionary divergence for *ORF* 1b, *S*, *N* and *M* genes for the retrieved sequences with other reference sequences. The lower right-check hand matrix of the table depicts the divergence and the upper left-check matrix of the matrix (blue colour) depicts the variation observed in the bootstrap replication

N gene	Alpha	Delta	Gamma	L_A	L_B	L_C	L_D	*M* gene	Alpha	Delta	Gamma	L_A	L_B	L_C	L_D
Alpha		0.15	0.09	0.10	0.08	0.08	0.09	Alpha		0.11	0.12	0.05	0.06	0.05	0.06
Delta	2.08		0.11	0.16	0.09	0.11	0.10	Delta	1.50		0.26	0.08	0.16	0.10	0.11
Gamma	1.57	1.49		0.08	0.08	0.09	0.08	Gamma	1.53	1.84		0.10	0.12	0.11	0.09
L_A	1.84	1.73	1.37		0.05	0.05	0.06	L_A	0.92	1.24	1.30		0.06	0.05	0.05
L_B	1.48	1.37	1.32	1.09		0.03	0.04	L_B	1.05	1.51	1.37	0.92		0.05	0.05
L_C	1.57	1.52	1.42	1.07	0.75		0.04	L_C	0.99	1.35	1.27	0.80	0.82		0.05
L_D	1.64	1.46	1.36	1.27	0.90	0.97		L_D	0.99	1.42	1.23	0.84	0.79	0.82	
ORF 1b	Alpha	Delta	Gamma	L_A	L_B	L_C	L_D	*ORF* 1a	Alpha	Delta	Gamma	L_A	L_B	L_C	L_D
Alpha		0.01	0.01	0.01	0.01	0.01	0.01	Alpha		0.02	0.02	0.01	0.02	0.02	0.03
Delta	0.70		0.01	0.02	0.01	0.01	0.01	Delta	1.32		0.03	0.02	0.03	0.03	0.04
Gamma	0.62	0.67		0.01	0.01	0.01	0.01	Gamma	1.14	1.33		0.02	0.03	0.02	0.04
L_A	0.61	0.69	0.60		0.01	0.01	0.01	L_A	1.22	1.01	1.30		0.02	0.02	0.04
L_B	0.60	0.70	0.65	0.54		0.01	0.01	L_B	1.26	1.42	1.41	1.19		0.01	0.02
L_C	0.58	0.69	0.62	0.53	0.50		0.01	L_C	1.35	1.41	1.44	1.19	0.97		0.03
L_D	0.60	0.67	0.61	0.53	0.50	0.52		L_D	1.26	1.27	1.39	1.09	0.90	1.03	
S gene	Alpha	Delta	Gamma	L_A	L_B	L_C	L_D	*E* gene	Alpha	Delta	Gamma	L_A	L_B	L_C	L_D
Alpha		0.02	0.02	0.03	0.03	0.03	0.02	Alpha		0.12	0.18	0.09	0.15	0.15	0.12
Delta	0.86		0.03	0.04	0.04	0.06	0.03	Delta	1.14		0.47	0.22	0.41	0.28	0.17
Gamma	1.14	0.96		0.04	0.05	0.06	0.04	Gamma	1.59	1.64		0.22	0.24	0.32	0.19
L_A	1.36	1.28	1.43		0.03	0.03	0.02	L_A	1.03	1.58	1.57		0.23	0.21	0.25
L_B	1.33	1.23	1.34	1.19		0.04	0.02	L_B	1.24	1.75	1.40	1.83		0.11	0.14
L_C	1.42	1.32	1.46	1.17	1.03		0.03	L_C	1.37	1.64	1.83	2.18	0.94		0.17
L_D	1.34	1.24	1.41	1.16	1.00	1.11		L_D	1.25	1.42	1.52	1.95	1.16	1.37	

ORF 1a, open reading frame 1a polyprotein; *ORF* 1b, ORF 1b polyprotein; *S*, spike glycoprotein; *N*, nuclocapsid phospoptotein; *M*, membrane glycoprotein; *E*, envelope protein

severity. It is still not understood as to why only certain CoVs can infect people.

There is a need of proactive surveillance of zoonotic infections in bats. Detection and identifications of such aetiological agents will provide leads for the development of diagnostic along with preparedness and readiness to deal with such emergent viruses thereby quickly containing them. The detection and identification of such viruses from bats also recommends cross-sectional antibody surveys (human and domestic animals) in localities where the viruses have been detected. Similarly, if epidemiological situation demands, evidence-based surveillance should also be conducted. There is a need of developing strong mechanisms for working jointly with various stakeholders such as wildlife, poultry, animal husbandry and human health departments.

In conclusion, our study showed detection of pathogenic CoVs in two species of Indian bats. Continuous active surveillance is required to identify the emerging novel viruses with epidemic potential.

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