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Ecological study of cave nectar bats reveals low risk of direct transmission of bat viruses to humans

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ABSTRACT

Bats are reservoirs of various viruses. The widely distributed cave nectar bat (*Eonycteris spelaea*) is known to carry both filoviruses and coronaviruses. However, the potential transmission of these bat viruses to humans is not fully understood. In this study, we tracked 16 *E. spelaea* bats in Mengla County, Yunnan Province, China, using miniaturized GPS devices to investigate their movements and potential contact with humans. Furthermore, to determine the prevalence of coronavirus and filovirus infections, we screened for the nucleic acids of the Měnglà virus (MLAV) and two coronaviruses (GCCDC1-CoV and HKU9-CoV) in anal swab samples taken from bats and for antibodies against these viruses in human serum samples. None of the serum samples were found to contain antibodies against the bat viruses. The GPS tracking results showed that the bats did not fly during the daytime and rarely flew to residential areas. The foraging range of individual bats also varied, with a mean

cumulative nightly flight distance of 25.50 km and flight speed of up to 57.4 km/h. Taken together, these results suggest that the risk of direct transmission of GCCDC1-CoV, HKU9-CoV, and MLAV from *E. spelaea* bats to humans is very low under natural conditions.

Keywords: Bats; *Eonycteris spelaea*; GPS tracking; GCCDC1-CoV; HKU9-CoV; Měnglà virus

INTRODUCTION

Many emerging infectious diseases (EIDs) that pose a threat to human health are zoonotic (Fauci & Morens, 2012; Jones et al., 2008). Bats are recognized as the natural reservoir of various viruses, such as the Marburg, Nipah, and Hendra viruses (Amman et al., 2020; Chua et al., 2002; Halpin et al., 2000; Towner et al., 2009). The ancestors of severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2,

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and Middle East respiratory syndrome coronavirus (MERS-CoV) are also thought to have originated in bats (Anthony et al., 2017; Ge et al., 2013; Hu et al., 2017; Temmam et al., 2022; Wacharapluesadee et al., 2021; Wong et al., 2020; Zhou et al., 2020b). With extensive surveillance and high-throughput nucleic acid sequencing, many novel bat viruses have been discovered; however, their potential transmission to humans is not yet fully understood (Chen et al., 2014; Tan et al., 2021).

The cave nectar bat (*Eonycteris spelaea*) is broadly distributed, with a geographical range from the Malay-Indonesian archipelago to southern China and South Asia (Francis et al., 2008). These bats are pollinators of many fruit trees and agricultural crops (Bumrungsri et al., 2013; Thavry et al., 2017) and can forage considerable distances (up to 38 km) from their roosting sites (Acharya et al., 2015). In China, *E. spelaea* bats are distributed in tropical regions, with the highest density found in Yunnan Province. Research has shown that *E. spelaea* bats can carry various coronaviruses (GCCDC1 coronavirus, GCCDC1-CoV; HKU9 coronavirus, HKU9-CoV) and filoviruses (Měnglà virus, MLAV) (Huang et al., 2016; Latinne et al., 2020; Luo et al., 2018; Yang et al., 2017, 2019). Both GCCDC1-CoV and HKU9-CoV belong to *Betacoronavirus* genus, and GCCDC1-CoV is a cross-family recombinant coronavirus with the P10 gene derived from orthoreovirus (Huang et al., 2016). GCCDC1-CoV, HKU9-CoV, and MLAV are also found in *Rousettus* and *Eonycteris* bats (Luo et al., 2018; Yadav et al., 2020). MLAV belongs to *Dianlovirus*, which is phylogenetically related to the Ebola and Marburg viruses of the *Filoviridae* family (Yang et al., 2019).

As bats primarily fly at night, studying their ecology can be extremely difficult. To overcome this limitation, solar-powered Global Positioning System (GPS) and very high frequency (VHF) tracking devices have been used to investigate the ecological traits of large arboreal fruit bats (Choden et al., 2019; Egert-Berg et al., 2018; Oleksy et al., 2017; Randhawa et al., 2020; Weller et al., 2016). For cave-dwelling bats, VHF and miniature non-rechargeable GPS devices have been used for tracking (Castle et al., 2015; Hurme et al., 2019), but are limited to medium-sized bats due to weight restrictions. Miniaturized GPS tracking devices based on the Global System for Mobile Communications (GSM) have also been developed for wild birds (Meng et al., 2020; Yu et al., 2017).

Risk assessment of viral infection has primarily focused on epidemiology (Dovich et al., 2019; Wang et al., 2018). We hypothesized that the ecological behavior of bats may also play a role in the transmission of viruses to humans. To test this hypothesis, we investigated the ecological behavior of *E. spelaea* bats in Mengla County, Yunnan Province, China, using GPS-GSM tracking devices, combined with study of bat virus epidemiology and local resident serology.

MATERIALS AND METHODS

Ethics statement

This study and related scientific field surveys were approved by the Guangdong Entomological Institute Administrative Panel (Approval No. GDEI-AE-2006001) and the Yunnan Institute of Endemic Diseases Control and Prevention

(Approval No. DLDXLL2013002). All animal and human sample collection protocols complied with the current laws of China.

Study sites

We investigated two colonies of *E. spelaea* bats in two different caves (Daoba Cave, E101.51356°, N21.795097°, 1 265 m above sea level (a.s.l.) and Tianshengqiao Cave, E101.374023°, N21.996787°, 655 m a.s.l.) located 44.3 km apart in Mengla County, Yunnan, China (Figure 1). Mengla has a population of more than 297 900 people, with major revenue from tourism and farming of tropical crops such as tea, bananas, and rubber (<https://www.ynml.gov.cn/index.shtml>). Both bat colonies are located close to residential and agricultural areas (Daoba Cave: 0.5 km, Tianshengqiao Cave: 2.7 km) and contain thousands of bats. The bats were captured with mist nets and temporarily restrained in a cotton bag. Only males and non-pregnant females were used (O'Mara et al., 2014; Sikes & The Animal Care and Use Committee of the American Society of Mammalogists, 2016; Sikes and Bryan II, 2016).

GPS-logger attachment

The GPS tracking device (GPS-logger) used in this study was developed by GSM (GSM-GPS, HQZN, Hunan Global Messenger Co., Ltd., China). The GPS transmitted time-stamped geolocation data to the receiver in the GPS-logger attached to each bat with a 92% accuracy within 10 m (Yu et al., 2017). The GPS-loggers collected positional data at pre-programmed intervals (between 1800h–0700h). The data, which included bat ID, date, time, longitude, latitude, speed, course, and altitude (Table 1), were then retrieved via the Short Message Service (SMS) of the GSM mobile phone network. All GPS-loggers were carefully inspected to ensure that they had no adverse effect on the bats.

Analyses of bat movement data

The GPS data were converted to Keyhole Markup Language (KML) files using the Tracker Client software, then imported into Google Earth Pro for display and analysis. Both the mean and range of cumulative distances flown each night and each hour by each bat were determined. The maximum straight-line distance between the caves and the most distant foraging site was also determined. Home ranges were estimated using the 100% (95%, 90%) minimum convex polygon (MCP) method and 95% (90%, 50%) fixed kernel density estimation (KDE) with a pre-defined reference bandwidth with the Home Range Tools (HRT) in ArcGIS® v10.2 (Baíllo & Chacón, 2021). Meteorological satellite data (October 2020) of the study areas were obtained from the Geospatial Data Cloud (<http://www.gscloud.cn/>), analyzed using ENVI v5.3 software for land use types, and mapped with ArcMap software. Based on the meteorological satellite data of Mengla in October 2020, land use types were classified as residential, forest, or agricultural. Average activity and average body temperature per hour of the bat populations were also determined.

Bat anal sample collection

Anal samples, which are considered better than nasal, pharyngeal, and blood samples for coronavirus detection

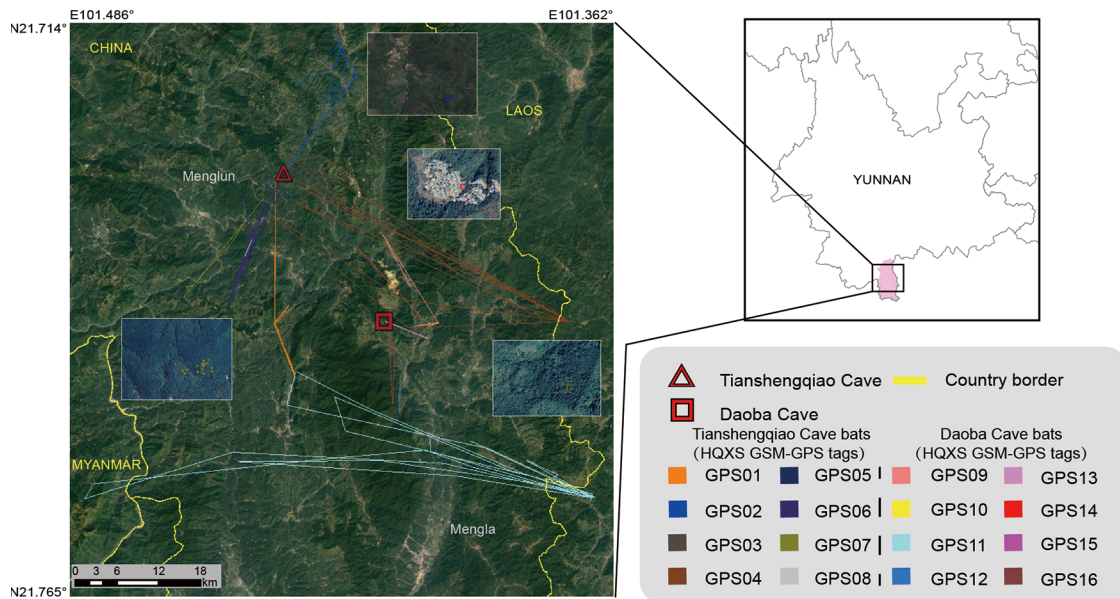


Figure 1 Flight map of 14 out of 16 bats tagged with GPS-loggers

Table 1 Summary of bat and GPS-logger data

Cave	GPS ID	Sex	GPS-logger weight (g)	Bat weight (g)	GPS-logger weight rate (%)	No. of nights tracked (n)	AGP/ADP
Tianshengqiao Cave	GPS01	M*	3.58	45	7.96	8	72/100
	GPS02	F*	3.53	40	8.83	7	48/92
	GPS03	F	3.6	63	5.71	—	—
	GPS04	M	3.61	83	4.35	6	40/77
	GPS05	M	3.66	75	4.88	6	45/77
	GPS06	M	3.67	82	4.48	5	34/61
	GPS07	M	3.64	78	4.67	6	35/76
	GPS08	M	3.63	80	4.54	3	10/21
Daoba Cave	GPS09	M	3.67	85.1	4.31	6	35/70
	GPS10	F	3.57	69.3	5.15	1	1/2
	GPS11	F	3.54	63.2	5.60	6	55/80
	GPS12	M	3.62	85.5	4.23	4	17/48
	GPS13	M	3.66	78.4	4.67	2	18/188
	GPS14	M	3.67	80.8	4.54	2	12/25
	GPS15	F	3.55	67.7	5.24	1	5/8
	GPS16	M	3.56	82	4.34	7	61/90

M: Male. F: Female. *: Subadult bats. AGP: Accumulated GPS points, which contain GPS information and other essential information; ADP: Accumulated data points, which contain only essential information. —: Not available.

(Zhou et al., 2020a), were collected as described previously (Li et al., 2005). All bats were released to their roosting site after sampling. Bat species were identified based on morphological characteristics and confirmed by cytochrome *b* (cyt *b*) gene sequencing (Luo et al., 2018). All samples were stored at -80°C until use.

Viral detection

RNA was extracted from the anal samples using a High Pure Viral RNA Kit (Roche, Switzerland). For detection of coronavirus RNA, a portion of the RNA-dependent RNA polymerase (RdRp) gene was amplified using a SuperScript III OneStep RT-PCR and Platinum Taq Enzyme Kit (Invitrogen,

USA) with family-specific degenerate semi-nested polymerase chain reaction (PCR), as described previously (Luo et al., 2018). Detection of filovirus RNA was performed following previous research (Yang et al., 2017). The PCR products were gel-purified and sequenced using the Sanger ABI-PRISM platform (Tsingke Biotechnology, China).

Serological tests

The GCCDC1-CoV nucleocapsid (N) protein (MG762691.1), HKU9-CoV N protein (MG762674.1), and MLAV N protein (KX371887.2) were expressed using the eukaryotic expression system in HEK293 cells (Supplementary Figure S1) (Li et al., 2019). The proteins were then used as antigens

to develop in-house anti-viral immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) kits. To perform ELISA, MaxiSorp Nunc-Immuno 96-well ELISA plates were coated (100 ng per well) overnight with a selected recombinant N protein. Human serum samples were collected by local clinics from 167 villagers who lived near the two study caves as previously study (Li et al., 2019). No personal identifying information was collected. The sera were used at a dilution of 1:20 and added to the wells for 1 h at 37 °C. Anti-human IgG horseradish peroxidase (HRP)-conjugated monoclonal secondary antibody (Kyab Biotech, China) was then used. The OD₄₅₀ values of wells were recorded to quantify antibodies against the selected viral N protein. For controls, we collected 96 serum samples from healthy participants in Wuhan, where the likelihood of people encountering bats is very low. The mean OD value of all negative serum samples plus three standard deviations was established as the cut-off value for positive serum (Li et al., 2019; Wang et al., 2018).

RESULTS

GPS-logger data

Sixteen cave nectar bats were tagged with GPS-loggers, including eight bats from Daoba Cave (five males and three females) and eight bats from Tianshengqiao Cave (six males and two females) (Figure 1; Table 1). The mean weight of the tagged GPS-loggers was less than 5.22% (3.61±0.05 g, min:

3.53 g, max: 3.67 g) of bat body weight (72.4±13.3 g, min: 40 g (subadult female), max: 85.5 g (adult male)). The longest working time of the GPS-loggers was eight nights (Table 1). Mean cumulative nocturnal flight distance was 25.76 km (min: 0.09 km, max: 132.18 km) for the Daoba Cave bats and 25.33 km (min: 0.03 km, max: 102.63 km) for the Tianshengqiao Cave bats (Table 2). Mean flight altitude was 1 000.19 m a.s.l. (min: 488 m, max: 1 833 m) for the Daoba Cave bats and 933.63 m a.s.l. (min: 599 m, max: 1 495 m) for the Tianshengqiao Cave bats (Table 2). Mean flying speed was 19.93 km/h (min: 1.5 km/h, max: 49.1 km/h) for the Daoba Cave bats and 19.84 km/h (min: 1.5 km/h, max: 57.4 km/h) for the Tianshengqiao Cave bats. The longest flight distance was recorded for a female Daoba Cave bat (GPS11), with a cumulative distance of 123.36 km in one night and 132.28 km the next night from its roost in Myanmar to a new roost in Laos (Table 2; Figure 1). One male Tianshengqiao Cave bat (GPS04) flew 89.9 km from Tianshengqiao Cave to its foraging ground in Laos every night for four consecutive nights and rested in Daoba Cave on its return on the fifth night (Table 2; Figure 1).

Spatial overlap of bat and human activities

Home ranges of individual bats varied from 0.06 and 2 905.13 km² based on 95% KDE and between 0.0005 and 780.05 km² based on 100% MCP estimation (Table 2). Home ranges were 1 528.38 km² (95% KDE) and 1 556.26 km² (100% MCP) for the eight Daoba Cave bats and 2 297.69 km² (95% KDE) and 1 504.49 km² (100% MCP) for the eight Tianshengqiao Cave

Table 2 Bat flight distance and speed.

Cave	GPS ID	Mean (min, max) nightly cumulative distance (km)	Mean (min, max) speed (km/h)	100% (95%, 90%) MCP (km ²)	95% (90%, 50%) KDE (km ²)	Maximum foraging distance (km)
Tianshengqiao Cave	GPS01	14.23 (0.54, 42.36)	17.63 (1.5, 38.3)	44.18 (12.56, 0.11)	42.47 (25.52, 5.53)	30.2
	GPS02	16.56 (0.68, 33.51)	18.21 (1.5, 43.2)	87.51 (49.66, 23.52)	181.20 (134.04, 30.41)	20.7
	GPS04	84.3 (62.33, 102.63)	22.75 (1.6, 45.5)	494.34 (489.8, 417.32)	2 337.78 (1 867.12, 541.07)	89.9
	GPS05	10.41 (0.27, 28.21)	20.92 (1.6, 57.4)	34.54 (0.66, 0.11)	27.5 (16.84, 4.18)	13.4
	GPS06	27.08 (7.65, 52.48)	22.29 (1.9, 37.1)	22.22 (12.07, 10.36)	303.92 (234.51, 46.03)	21.5
	GPS07	14.95 (0.23, 35.29)	17.83 (1.5, 35.9)	10.63 (8.23, 0.45)	94.23 (59.99, 12.15)	23.2
	GPS08	4.97 (0.03, 11.75)	12.80 (4.4, 24.9)	0.09 (0.09, 0.006)	4.61 (3.3, 0.74)	14.4
	GPS09	7.75 (0.69, 18.98)	12.40 (1.9, 31.9)	26.77 (11.95, 0.33)	29.63 (23.31, 6.33)	12.7
Daoba Cave	GPS10	14.84 (14.84, 14.84)	33.20 (33.2, 33.2)	—	—	14.8
	GPS11	90.71 (62.97, 132.18)	27.89 (1.5, 49.1)	780.05 (431.39, 277.97)	2 905.13 (2 196.41, 504.88)	51.8
	GPS12	6.36 (0.09, 18.84)	22.97 (9.5, 32.6)	0.54 (0.54, 0.05)	9.32 (6.51, 1.59)	13.3
	GPS13	17.12 (7.12, 27.13)	15.31 (1.9, 34.2)	0.67 (0.67, 0.39)	43.96 (34.23, 7.28)	6.9
	GPS14	17.2 (5.65, 28.74)	15.08 (2.3, 27.3)	0.40 (0.40, 0.001)	112.46 (80.08, 18.52)	12.1
	GPS15	13.7 (13.7, 13.7)	3.75 (3.4, 4.1)	0.0005 (0.0005, 0.0005)	0.06 (0.054, 0.014)	13.1
	GPS16	4.79 (0.37, 18.85)	14.93 (1.5, 40.4)	4.88 (0.67, 0.07)	12.75 (8.09, 1.75)	11.9

MCP: Minimum convex polygon. KDE: Kernel density estimates. —: Not available.

bats. The overlapping home range area between the Daoba Cave and Tianshengqiao Cave bats was 286.77 km² (95% KDE) and 359.79 km² (100% MCP) (Figures 2, 3). The area around Daoba Cave was mainly forest, with the main activity areas being farmland (70.89%), forest (26.67%), and residential (1.44%) (Figure 3). The area around Tianshengqiao Cave was mainly farmland, with the main activity areas being forest (76.06%), farmland (21.83%), and residential (2.11%) (Figure 3). Overall, the main activity areas of all GPS-logger tagged bats in Mengla were farmland (56.64%), forest (42.72%), and residential (1.64%) (Figure 3).

Time separation between bat and human activities

The first peak in activity (motion collected by GPS-loggers) of the Daoba Cave bats occurred from 1800h to 1930h, with second, third, and fourth peaks in activity occurring at about 0030h, 0230h, and 0630h, respectively (Figure 4A). The first peak in activity of the Tianshengqiao Cave bats occurred from 1800h to 2030h, with second, third, and fourth peaks in activity occurring at about 2230h, 0130h, and 0630h, respectively (Figure 4B). These results were positively correlated with average activity per hour and temperature around the bat bodies. During the daytime (0730h–1730h), the bats rested in

the caves. The Daoba Cave bats left the cave about 15 min after sunset, while the Tianshengqiao Cave bats left the cave about 10 min after sunset. As human activity in the agricultural areas mainly occurred during the daytime, there was little overlap in time between human and bat activities.

Prevalence of viruses in *E. spelaea*

The anal swab samples of bats from the Daoba and Tianshengqiao caves were collected from January to December 2019 (Figure 1). Seventeen (12.8%) of the 133 samples were determined to be positive for GCCDC1-CoV or closely related viruses based on RT-PCR targeting the RdRp gene. The positive rates for GCCDC1-CoV varied with sampling time and site. GCCDC1-CoV was not detected in Daoba Cave bats in December but was relatively prevalent in June (5 of 39, 12.8%) and January (9 of 30, 30.0%). Three (10.3%) of the 30 Tianshengqiao Cave bats were positive for GCCDC1-CoV in December (Table 3). HKU9-CoV and MLAV were not detected in any *E. spelaea* bats examined in this study (Table 3).

Seroprevalence of viruses in humans

A total of 167 human serum samples from three villages near

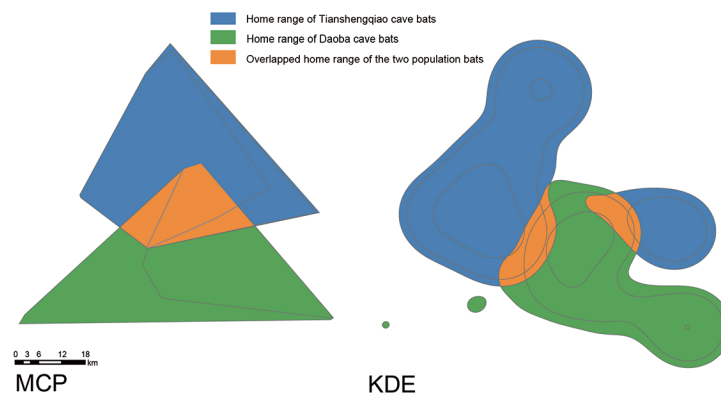


Figure 2 Simulation map of bat home range. Left: bat home range simulated by MCP

Right: Bat home range simulated by KDE. Blue: Home range of Tianshengqiao Cave bats. Green: Home range of Daoba Cave bats. Orange: Overlapping home range of both bat colonies.

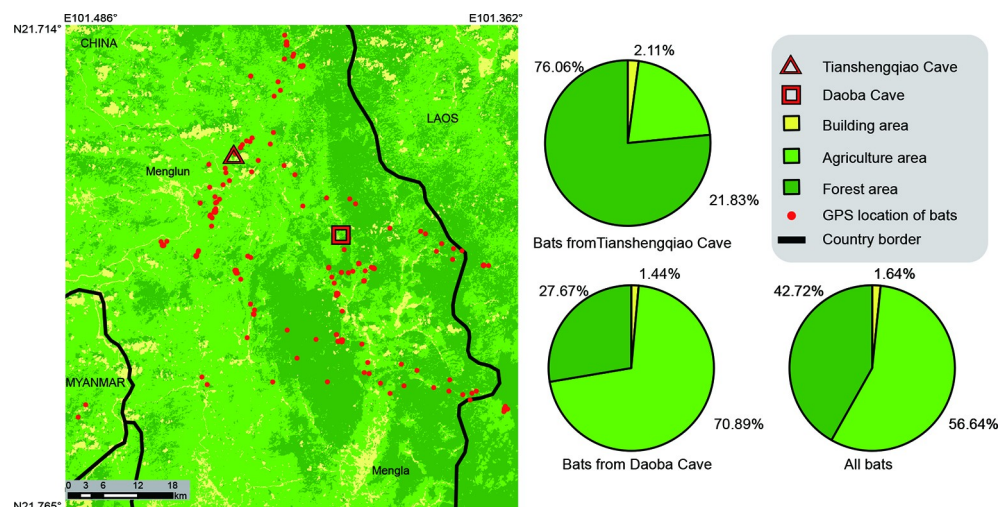


Figure 3 GPS localization of bats in different areas (left) and percentage of two bat colonies in different areas

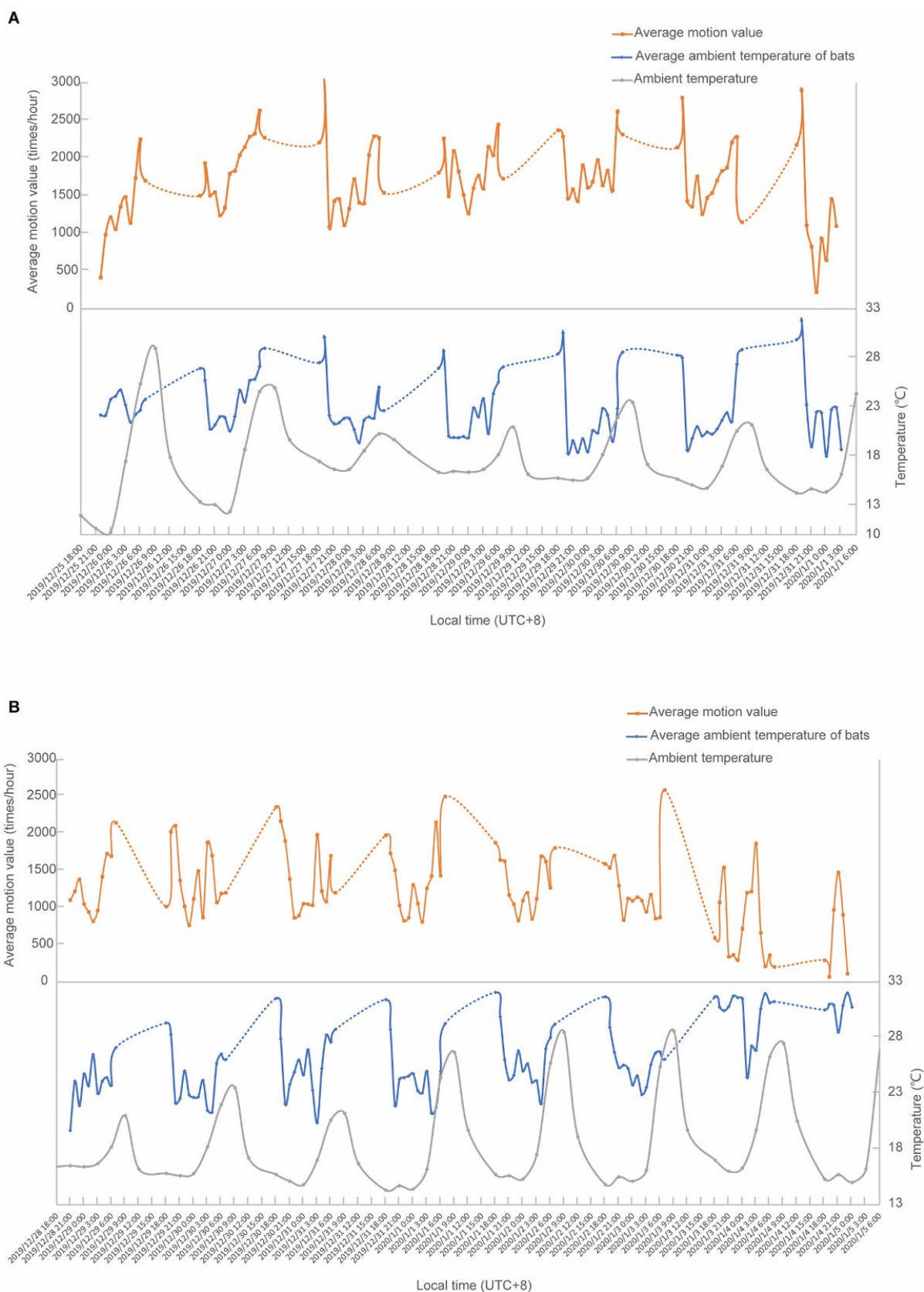


Figure 4 Temporal pattern of bat activity

Daoba Cave were collected and screened by ELISA using the N proteins of GCCDC1-CoV, HKU9-CoV, and MLAV as antigens, with the cutoff value determined based on healthy serum samples previously collected in Wuhan (Wang et al., 2018). Results showed that all serum samples were negative (Figure 5).

DISCUSSION

The incidence of EIDs has increased over the last 50 years (Jones et al., 2008). More than 70% of EIDs are of animal origin and bats are considered one of the most common hosts of diverse viruses (Allen et al., 2017; Olival et al., 2017). Global environmental changes, such as deforestation for agricultural purposes, are presumed to be a major cause of EIDs (Loh et al., 2015). Thus, understanding the ecological overlap between viral prevalence and human populations is crucial for controlling zoonotic infections.

In this study, we performed longitudinal surveillance to monitor the presence of GCCDC1-CoV, HKU9-CoV, and MLAV in *E. spelaea* bats, with only GCCDC1-CoV detected. HKU9-CoV is specific to *Rousettus leschenaultia* bats, while MLAV can infect both *R. leschenaultia* and *E. spelaea* bats (Gao et al., 2019; Yang et al., 2017). Our failure to detect MLAV in the bats may be due to its lung tissue tropism and that anal samples rather than lung tissue samples were used.

Our results showed that *E. spelaea* bats have a broad home range in tropical areas, including Laos, Myanmar, and Yunnan in China. Several models have been used to estimate bat home ranges, including the MCP, KDE, and low convex hull

(LoCoH) models (Boyle et al., 2009; Choden et al., 2019; Fahr et al., 2015). The LoCoH model was not used here as it is mainly applied to analyze cliffs, water, and unforested areas not inhabited by bats (Getz et al., 2007). For risk assessment of bat virus spillover, both maximum range of transmission and high-risk areas need to be considered. The MCP model generates home ranges based on peripheral activity sites, thus reflecting the boundary range of bats (Hayne, 1949; Mohr, 1947). The KDE model describes spatial utilization by location, thus reflecting the hot spot areas of bats (Worton, 1989). Therefore, both MCP and KDE were used to estimate the home ranges of the *E. spelaea* bats in this study.

Eonycteris spelaea is a cave-dwelling colony species that occupies a variety of habitats, including orchards and primary and secondary forests (Heideman and Heaney, 1989; KITCHENER et al., 1990). In Mengla, *E. spelaea* bats are rarely found in residential areas, but are present in agricultural and forested areas (Mishra et al., 2020). As the Daoba and Tianshengqiao cave bats do not normally fly to residential areas, the possibility of transmitting viruses to humans in these areas is very low. However, they may spread viruses in agricultural areas where both human and bat activities occur.

GPS tracking revealed four major movement times of both the Daoba and Tianshengqiao cave bats. Similar to bats in other regions, these bats do not leave their roosts after their dawn return (Acharya et al., 2015). A previous study in Thailand showed that *E. spelaea* activity varies with the presence of different plants in different seasons (Bumrungsri et al., 2013; Srithongchuay et al., 2008). The Mengla residents are mainly engaged in agricultural activity between sunrise

Table 3 Virus positive rates in *E. spelaea* bats

Date	Sampling site	Virus RNA prevalence		
		GCCDC1-CoV	HKU9-CoV	MLAV
2019/1/13	Daoba Cave	9/30 (30.0%)	0/30 (0.0%)	0/30 (0.0%)
2019/6/4	Daoba Cave	5/39 (12.8%)	0/39 (0.0%)	0/39 (0.0%)
2019/12/25	Daoba Cave	0/35 (0.0%)	0/35 (0.0%)	0/35 (0.0%)
2019/12/28	Tianshengqiao Cave	3/29 (10.3%)	0/29 (0.0%)	0/29 (0.0%)
Total	–	17/133 (12.8%)	0/133 (0.0%)	0/133 (0.0%)

GCCDC1-CoV: GCCDC1 coronavirus; HKU9-CoV: HKU9 coronavirus; MLAV: Měnglà virus. –: Not available.

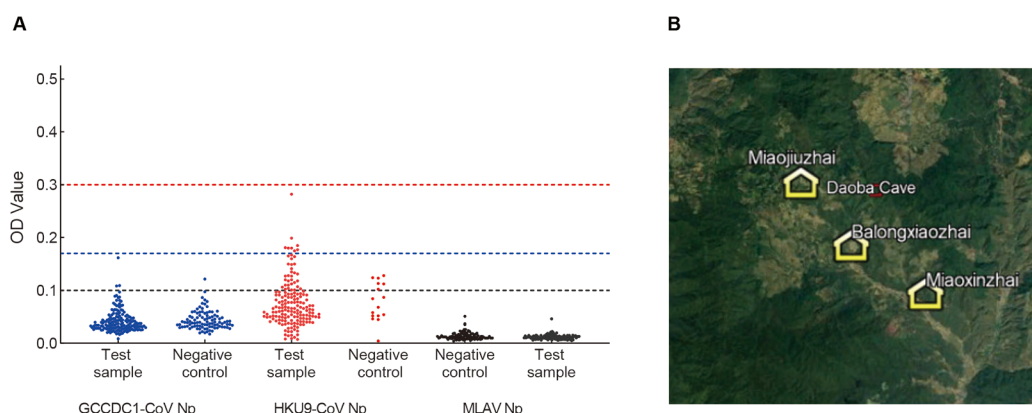


Figure 5 Serological test results of people residing near bat colonies

A: Seroprevalence of GCCDC1-CoV, HKU9-CoV, and MLAV in individuals examined. B: Locations of serum sampling sites.

and sunset. As bats rarely fly during the daytime, this separation in bat and human activity reduces the risk of direct transmission of bat viruses to humans (Plowright et al., 2017).

Mengla County borders Laos in the east, south, and southwest, and Myanmar across the Lancang River in the northwest. The area has a tropical rainforest climate, which is suitable for *E. spelaea* survival. In this study, we found that the Mengla *E. spelaea* bats tended to forage southward. Our ecological and serological results indicated that the risk of direct transmission of *E. spelaea* viruses to humans is low due to limited overlap in spatial and temporal activity, thus restricting zoonotic spillover.

Our study has several limitations. First, the number of human serum samples and GPS-tracked bats was relatively small. Second, the potential intermediate hosts of bat viruses were not investigated. Thus, in addition to epidemiological and ecological studies, the entire transmission chain of zoonoses should be investigated in future studies to minimize EIDs infection.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Field surveys and specimen collection protocols were approved by the Guangdong Entomological Institute Administrative Panel (GDEI- AE- 2006001) and Yunnan Institute of Endemic Diseases Control and Prevention (DLDXLL2013002).

DATA AVAILABILITY

The meteorological satellite data of the study areas are available at <http://www.gscloud.cn/>.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

K.Z., X.L.Y., L.B.Z., and Z.L.S. designed the study; K.Z., X.L.Y., L.B.Z., F.Y., R.D.J., Y.Z.Z., and X.Y.H. collected the samples; K.Z., W.Z., B.L., Y.L., and S.Z.X. performed experiments; K.Z. and Y.L.Y. analyzed the data; K.Z., X.L.Y., L.B.Z., and Z.L.S. wrote the manuscript. All authors read and approved the final version of the manuscript.

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REFERENCES

Acharya PR, Racey PA, Sothibandhu S, Bumrungsri S. 2015. Home-range and foraging areas of the dawn bat *Eonycteris spelaea* in agricultural areas of thailand. *Acta Chiropterologica*, 17(2): 307–319.

Allen T, Murray KA, Zambrana-Torrel C, Morse SS, Rondinini C, Di Marco M, et al. 2017. Global hotspots and correlates of emerging zoonotic

diseases. *Nature Communications*, 8(1): 1124.

Amman BR, Bird BH, Bakarr IA, Bangura J, Schuh AJ, Johnny J, et al. 2020. Isolation of Angola-like marburg virus from egyptian roussette bats from West Africa. *Nature Communications*, 11(1): 510.

Anthony SJ, Gilardi K, Menachery VD, Goldstein T, Ssebide B, Mbabazi R, et al. 2017. Further evidence for bats as the evolutionary source of middle east respiratory syndrome coronavirus. *mBio*, 8(2): e00373–17.

Baillio A, Chacón JE. 2021. Statistical outline of animal home ranges: an application of set estimation. *Handbook of Statistics*, 44: 3–37.

Boyle SA, Lourenço WC, da Silva LR, Smith AT. 2009. Home range estimates vary with sample size and methods. *Folia Primatologica*, 80(1): 33–42.

Bumrungsri S, Lang DC, Harrower C, Sriparaya E, Kitpipit K, Racey PA. 2013. The dawn bat, *Eonycteris spelaea* dobson (chiroptera: pteropodidae) feeds mainly on pollen of economically important food plants in Thailand. *Acta Chiropterologica*, 15(1): 95–104.

Castle KT, Weller TJ, Cryan PM, Hein CD, Schirmacher MR. 2015. Using sutures to attach miniature tracking tags to small bats for multimonth movement and behavioral studies. *Ecology and Evolution*, 5(14): 2980–2989.

Chen L, Liu B, Yang J, Jin Q. 2014. DBatVir: the database of bat-associated viruses. *Database (Oxford)*, 2014: bau021.

Choden K, Ravon S, Epstein JH, Hoem T, Furey N, Gely M, et al. 2019. Pteropus lylei primarily forages in residential areas in Kandal, Cambodia. *Ecology and Evolution*, 9(7): 4181–4191.

Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, et al. 2002. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes and Infection*, 4(2): 145–151.

Dovih P, Laing ED, Chen YH, Low DHW, Ansil BR, Yang X, et al. 2019. Filovirus-reactive antibodies in humans and bats in Northeast India imply zoonotic spillover. *PLOS Neglected Tropical Diseases*, 13(10): e0007733.

Egert-Berg K, Hurme ER, Greif S, Goldstein A, Harten L, Herrera MLG, et al. 2018. Resource ephemerality drives social foraging in bats. *Current Biology*, 28(22): 3667–3673.e5.

Fahr J, Abedi-Lartey M, Esch T, Machwitz M, Suu-Ire R, Wikelski M, et al. 2015. Pronounced seasonal changes in the movement ecology of a highly gregarious central-place forager, the african straw-coloured fruit bat (*Eidolon helvum*). *PLoS One*, 10(10): e0138985.

Fauci AS, Morens DM. 2012. The perpetual challenge of infectious diseases. *New England Journal of Medicine*, 366(5): 454–461.

Francis C, Rosell-Ambal G, Tabaranza B, Carino P, Helgen K, Molur S, et al. 2008. *Eonycteris Spelaea* (The IUCN Red List of Threatened Species 2008: e. T7787A12850087.). International Union for Conservation of Nature.

Gao FL, Liu XW, Du ZG, Hou H, Wang XY, Wang FL, et al. 2019. Bayesian phylodynamic analysis reveals the dispersal patterns of tobacco mosaic virus in China. *Virology*, 528: 110–117.

Ge XY, Li JL, Yang XL, Chmura AA, Zhu GJ, Epstein JH, et al. 2013. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature*, 503(7477): 535–538.

Getz WM, Fortmann-Roe S, Cross PC, Lyons AJ, Ryan SJ, Wilms CC. 2007. LoCoH: nonparametric kernel methods for constructing home ranges and utilization distributions. *PLoS One*, 2(2): e207.

Halpin K, Young PL, Field HE, Mackenzie JS. 2000. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *Journal of General Virology*, 81(8): 1927–1932.

Hayne DW. 1949. Calculation of size of home range. *Journal of Mammalogy*, 30(1): 1–18.

Heideman PD, Heaney LR. 1989. Population biology and estimates of abundance of fruit bats (Pteropodidae) in Philippine submontane rainforest. *Journal of Zoology*, 218(4): 565–586.

- Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, et al. 2017. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog*, **13**(11): e1006698.
- Huang CP, Liu WJ, Xu W, Jin T, Zhao YZ, Song JD, et al. 2016. A bat-derived putative cross-family recombinant coronavirus with a reovirus gene. *PLoS Pathog*, **12**(9): e1005883.
- Hurme E, Gurarie E, Greif S, Herrera MLG, Flores-Martínez JJ, Wilkinson GS, et al. 2019. Acoustic evaluation of behavioral states predicted from GPS tracking: a case study of a marine fishing bat. *Movement Ecology*, **7**: 21.
- Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. 2008. Global trends in emerging infectious diseases. *Nature*, **451**(7181): 990–993.
- Kitchener DJ, Gunnell A, Maharadatunkamsi. 1990. Aspects of the feeding biology of fruit bats (Pteropodidae) on Lombok Island, Nusa Tenggara, Indonesia. *Mammalia*, **54**(4): 561–578.
- Latinné A, Hu B, Olival KJ, Zhu GJ, Zhang LB, Li HY, et al. 2020. Origin and cross-species transmission of bat coronaviruses in China. *Nature Communications*, **11**(1): 4235.
- Li HY, Mendelsohn E, Zong C, Zhang W, Hagan E, Wang N, et al. 2019. Human-animal interactions and bat coronavirus spillover potential among rural residents in Southern China. *Biosafety and Health*, **1**(2): 84–90.
- Li WD, Shi ZL, Yu M, Ren WZ, Smith C, Epstein JH, et al. 2005. Bats are natural reservoirs of SARS-like coronaviruses. *Science*, **310**(5748): 676–679.
- Loh EH, Zambrana-Torrel C, Olival KJ, Bogich TL, Johnson CK, Mazet JAK, et al. 2015. Targeting transmission pathways for emerging zoonotic disease surveillance and control. *Vector-Borne and Zoonotic Diseases*, **15**(7): 432–437.
- Luo Y, Li B, Jiang RD, Hu BJ, Luo DS, Zhu GJ, et al. 2018. Longitudinal surveillance of betacoronaviruses in fruit bats in Yunnan province, China during 2009–2016. *Virologica Sinica*, **33**(1): 87–95.
- Meng FJ, Wang X, Batbayar N, Natsagdorj T, Davaasuren B, Damba I, et al. 2020. Consistent habitat preference underpins the geographically divergent autumn migration of individual Mongolian common shelducks. *Current Zoology*, **66**(4): 355–362.
- Mishra PK, Rai A, Rai SC. 2020. Land use and land cover change detection using geospatial techniques in the Sikkim Himalaya, India. *The Egyptian Journal of Remote Sensing and Space Science*, **23**(2): 133–143.
- Mohr CO. 1947. Table of equivalent populations of North American small mammals. *The American Midland Naturalist*, **37**(1): 223–249.
- O'Mara MT, Wikelski M, Dechmann DKN. 2014. 50 years of bat tracking: device attachment and future directions. *Methods in Ecology and Evolution*, **5**(4): 311–319.
- Oleksy R, Giuggioli L, McKetterick TJ, Racey PA, Jones G. 2017. Flying foxes create extensive seed shadows and enhance germination success of pioneer plant species in deforested Madagascan landscapes. *PLoS One*, **12**(9): e0184023.
- Olival KJ, Hosseini PR, Zambrana-Torrel C, Ross N, Bogich TL, Daszak P. 2017. Host and viral traits predict zoonotic spillover from mammals. *Nature*, **546**(7660): 646–650.
- Plowright RK, Parrish CR, McCallum H, Hudson PJ, Ko AI, Graham AL, et al. 2017. Pathways to zoonotic spillover. *Nature Reviews Microbiology*, **15**(8): 502–510.
- Randhawa N, Bird BH, VanWormer E, Sijali Z, Kilonzo C, Mwigwa A, et al. 2020. Fruit bats in flight: a look into the movements of the ecologically important *Eidolon helvum* in Tanzania. *One Health Outlook*, **2**(1): 16.
- Sikes RS, The Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy*, **97**(3): 663–688.
- Sikes RS, Bryan II JA. 2016. Institutional animal care and use committee considerations for the use of wildlife in research and education. *ILAR Journal*, **56**(3): 335–341.
- Srithongchuay T, Bumrungsri S, Sripao-Raya E. 2008. The pollination ecology of the late-successional tree, *Oroxylum indicum* (Bignoniaceae) in Thailand. *Journal of Tropical Ecology*, **24**(5): 477–484.
- Tan CW, Yang X, Anderson DE, Wang LF. 2021. Bat virome research: the past, the present and the future. *Current Opinion in Virology*, **49**: 68–80.
- Temmam S, Vongphayloth K, Baquero E, Munier S, Bonomi M, Regnault B, et al. 2022. Bat coronaviruses related to SARS-CoV-2 and infectious for human cells. *Nature*, **604**(7905): 330–336.
- Thavry H, Cappelle J, Bumrungsri S, Thona L, Furey NM. 2017. The diet of the cave nectar bat (*Eonycteris spelaea* dobson) suggests it pollinates economically and ecologically significant plants in southern cambodia. *Zoological Studies*, **56**: e17.
- Towner JS, Amman BR, Sealy TK, Carroll SAR, Comer JA, Kemp A, et al. 2009. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathogens*, **5**(7): e1000536.
- Wacharapluesadee S, Tan CW, Maneeorn P, Duengkae P, Zhu F, Joyjinda Y, et al. 2021. Evidence for SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia. *Nature Communications*, **12**(1): 972.
- Wang N, Li SY, Yang XL, Huang HM, Zhang YJ, Guo H, et al. 2018. Serological evidence of bat SARS-related coronavirus infection in humans, China. *Virologica Sinica*, **33**(1): 104–107.
- Weller TJ, Castle KT, Liechti F, Hein CD, Schirmacher MR, Cryan PM. 2016. First direct evidence of long-distance seasonal movements and hibernation in a Migratory Bat. *Scientific Reports*, **6**: 34585.
- Wong G, Bi YH, Wang QH, Chen XW, Zhang ZG, Yao YG. 2020. Zoonotic origins of human coronavirus 2019 (HCoV-19/SARS-CoV-2): why is this work important?. *Zoological Research*, **41**(3): 213–219.
- Worton BJ. 1989. Kernel methods for estimating the utilization distribution in home - range studies. *Ecology*, **70**(1): 164–168.
- Yadav PD, Shete-Aich A, Nyayanit DA, Pardeshi P, Majumdar T, Balasubramanian R, et al. 2020. Detection of coronaviruses in *Pteropus* & *Rousettus* species of bats from different States of India. *Indian Journal of Medical Research*, **151**(2-3): 226–235.
- Yang XL, Tan CW, Anderson DE, Jiang RD, Li B, Zhang W, et al. 2019. Characterization of a filovirus (Mênglà virus) from *Rousettus* bats in China. *Nature Microbiology*, **4**(3): 390–395.
- Yang XL, Zhang YZ, Jiang RD, Guo H, Zhang W, Li B, et al. 2017. Genetically diverse filoviruses in *Rousettus* and *Eonycteris* spp. Bats, China, 2009 and 2015. *Emerging Infectious Diseases*, **23**(3): 482–486.
- Yu H, Wang X, Cao L, Zhang L, Jia Q, Lee H, et al. 2017. Are declining populations of wild geese in China 'prisoners' of their natural habitats?. *Current Biology*, **27**(10): R376–R377.
- Zhou H, Chen X, Hu T, Li J, Song H, Liu YR, et al. 2020a. A novel bat coronavirus closely related to SARS-CoV-2 contains natural insertions at the S1/S2 cleavage site of the spike protein. *Current Biology*, **30**(11): 2196–2203.e3.
- Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. 2020b. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, **579**(7798): 270–273.

Ecological study of cave nectar bats reveals low risk of direct transmission of bat viruses to humans

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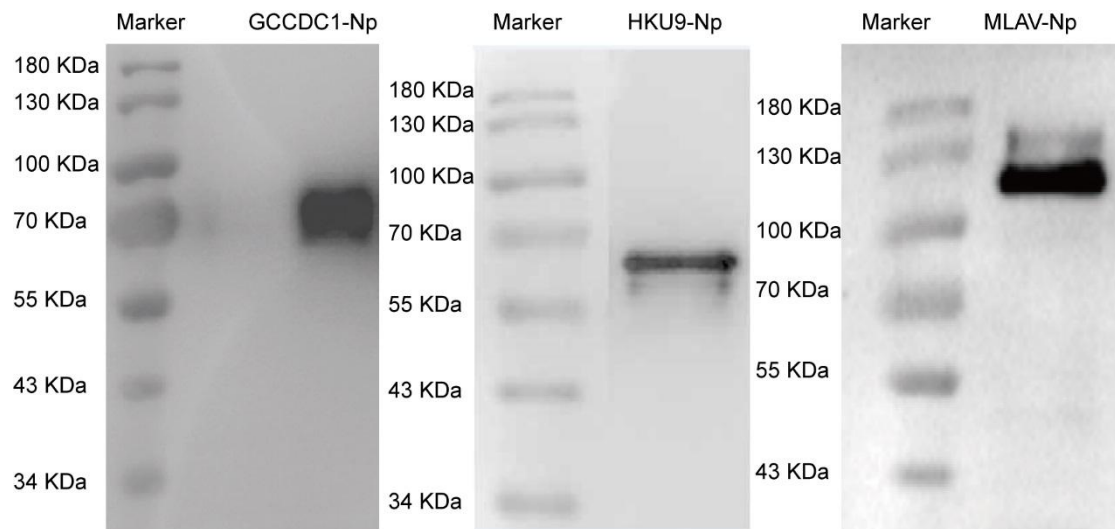
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Supplementary Materials



Supplementary Figure S1. Expression and purification of N protein of GCCDC1-CoV, HKU9-CoV, and MLAV.