

Establishment of a Captive Cave Nectar Bat (*Eonycteris spelaea*) Breeding Colony in Singapore

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Bats are known natural reservoirs of several highly pathogenic zoonotic viruses, including Hendra virus, Nipah virus, rabies virus, SARS-like coronaviruses, and suspected ancestral reservoirs of SARS-CoV-2 responsible for the ongoing COVID-19 pandemic. The capacity to survive infections of highly pathogenic agents without severe disease, together with many other unique features, makes bats an ideal animal model for studying the regulation of infection, cancer, and longevity, which is likely to translate into human health outcomes. A key factor that limits bat research is lack of breeding bat colonies. To address this need, a captive bat colony was established in Singapore from 19 wild-caught local cave nectar bats. The bats were screened for specific pathogens before the start of captive breeding. Custom-made cages and an optimized diet inclusive of Wombaroo dietary formula, liquid diet, and supplement of fruits enabled the bats to breed prolifically in our facility. Cages are washed daily and disinfected once every fortnight. Bats are observed daily to detect any sick bat or abnormal behavior. In addition, bats undergo a thorough health check once every 3 to 4 mo to check on their overall wellbeing, perform sampling, and document any potential pregnancy. The current colony houses over 80 bats that are successfully breeding, providing a valuable resource for research in Singapore and overseas.

Abbreviations: AVS, Animal & Veterinary Service; *E. spelaea*, *Eonycteris spelaea*; MERS-CoV, Middle East respiratory syndrome-related coronavirus; NLARF, National Large Animal Research Facility; NParks, National Parks Board; SARS-CoV, Severe acute respiratory syndrome coronavirus

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Introduction

Bats are known reservoirs of several zoonotic pathogens that pose a threat to human and animal health. These pathogens include Marburg virus, Hendra virus, Nipah virus, and rabies virus.⁷ In addition, they are likely the ancestral reservoirs for Severe Acute Respiratory Syndrome – Coronavirus (SARS-CoV 1 and SARS-CoV-2), Middle East Respiratory Syndrome coronavirus (MERS-CoV), and Ebola virus.^{5,7,29} Bats' immunologic response to infection from viruses that are highly pathogenic in other mammals, the lack of tumorigenesis, and their longevity make bats a popular model for infectious disease research.^{1,6,16} Conducting in vitro studies creates a continuous need to collect samples from bats. However, significant challenges exist when sampling wild bats. In addition to disturbing the bat's natural habitat, wild bats may introduce numerous confounding variables, such as large diversity in immune response, unknown disease status, and pathogen exposure history, which may negatively impact the study.^{2,15} To address some of these challenges, we established a breeding colony of *Eonycteris spelaea* in Singapore for research.

Eonycteris spelaea, also known as cave nectar bat, belongs to the subfamily *Rousettinae* in the family *Pteropodida*.²⁵ *E. spelaea* is an excellent choice for the establishment of a breeding colony for several reasons. They are classified as 'least concern' on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species.²⁵ In addition, these bats roost in large dense colonies within a small area, and we posit that these bats will be able to adapt quickly to our proposed colony setup (Figure 1). Also, the genome and transcriptome have already been well characterized.²⁶ Furthermore, the availability of a continuous source of samples from the colony will help to address gaps through in vivo functional analysis of genes or pathways of interest. Finally, the *E. spelaea* roosts in Singapore are accessible for researchers to safely trap the bats.

Here, we describe the establishment and subsequent reproductive success of a captive colony of *E. spelaea*. To our knowledge, this is the first nectivorous captive, reproductive bat colony in Asia.

Methods

Animals. Animal ethics approval for the study was granted by SingHealth Institutional Animal Care and Use Committee (IACUC; Permit # 2015/SHS/1088). Regulatory approval for the trapping of bats was granted by the National Parks Board, Singapore (NParks; Permit# NP/RP15-087). Bats were captured between 1900 and 2100 h at 2 sites in Singapore using elevated mist nets supported by a pair of 3.00 m to 3.50 m telescopic

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Figure 1. *Eonycteris spelaea* roosting in the artificial housing.

poles. Trapped bats were removed from mist nets, placed into custom-made, breathable opaque cotton mesh holding bags, and inspected for age and sex. Bats weighing above 47.0 g with a visible distension of the stomach were suspected to be pregnant and released (Figure 2). Juvenile bats were also released. Bat mites were removed using blunt-end forceps, and any physical wounds were treated with antiseptic cream (Spectracon, New Delhi, India). Wild caught bats were quarantined for 1 mo in a separate cage before introduction to colony. All personnel wore Tyvek suits with safety goggles, N95 masks, head lamp, and bite-resistant leather gloves over nitrile gloves when trapping bats.

Care and Welfare

Animal housing. The bat facility includes an air-conditioned anteroom (3.00 m by 2.65 m) connected to an Animal Biosafety Level-1 (ABSL-1) lab (3.00 m by 2.98 m) that is connected to the bat holding area. The holding area (7.90 m by 5.66 m) is sheltered, naturally ventilated, exposed to natural photoperiod, and enclosed by wire mesh to prevent the entry of rodents, birds, and wild animals.

Custom cages were designed and fabricated (Steelmatic, Singapore) (Figure 3). Cages were 100 cm long, 100 cm wide, and 183 cm high. The front, top, and bottom panels of the cages were made of heavy-duty stainless steel wire mesh with no sharp edges. The top panel allowed the bats to hang freely and personnel to hang the feeding pan. The wire mesh has a 1-cm space opening, and the 2 side metal sheets are retractable. When removed, additional cages can be added on either side or on the back of the cage, allowing the bats to roam freely between adjoining cages. A removable metal collection pan was

also incorporated under the bottom wire mesh panel to allow for the daily removal of waste. Hessian fabric was hung in the cage supported by a tree branch to allow bats to roost together. Outside the cage, an additional drape was used to cover one end of the cage for extra privacy and shade (Figure 4). Each enclosure consisted of 2 cages joined back-to-back and could house a maximum of 25 bats. This arrangement provided ample space for the bats to fly within the enclosure.

Diet. *E. spelaea*'s natural diet consists primarily of nectar and pollen.¹⁴ To replicate their natural diet, water, milk powder, powder pollen, and a commercially available diet (Wombaroo Lorikeet and Honeyeater Food, Glen Osmond, SA, Australia) were provided. Fresh fruit was also provided based on seasonal availability (Table 1). The bats were fed once daily in the evening and feed was left overnight.

Sanitation. Leftover feed from the previous evening and waste were removed. External cage surfaces were washed with water every morning. Bats were transferred to clean cages every 2 wks for cage disinfection. Cages were disinfected with 1% Virkon for 30 min of contact time followed by a rinse with water. Bat holding bags were washed after each use with detergent and disinfected using 1% Virkon for 30 min of contact time followed by a rinse with water.

Veterinary Check and surveillance. Bats were monitored daily from outside the cage for any sign of isolation or abnormal behavior. Bats that appeared weak and emaciated were assessed by the facility veterinarian. Thorough medical health checks were performed once every 3 to 4 mo to collect samples (oral, rectal, and body swabs, and blood), detect physical injury, perform morphometric measurement, identify pregnant bats, and microchip newborn pups that had been weaned.

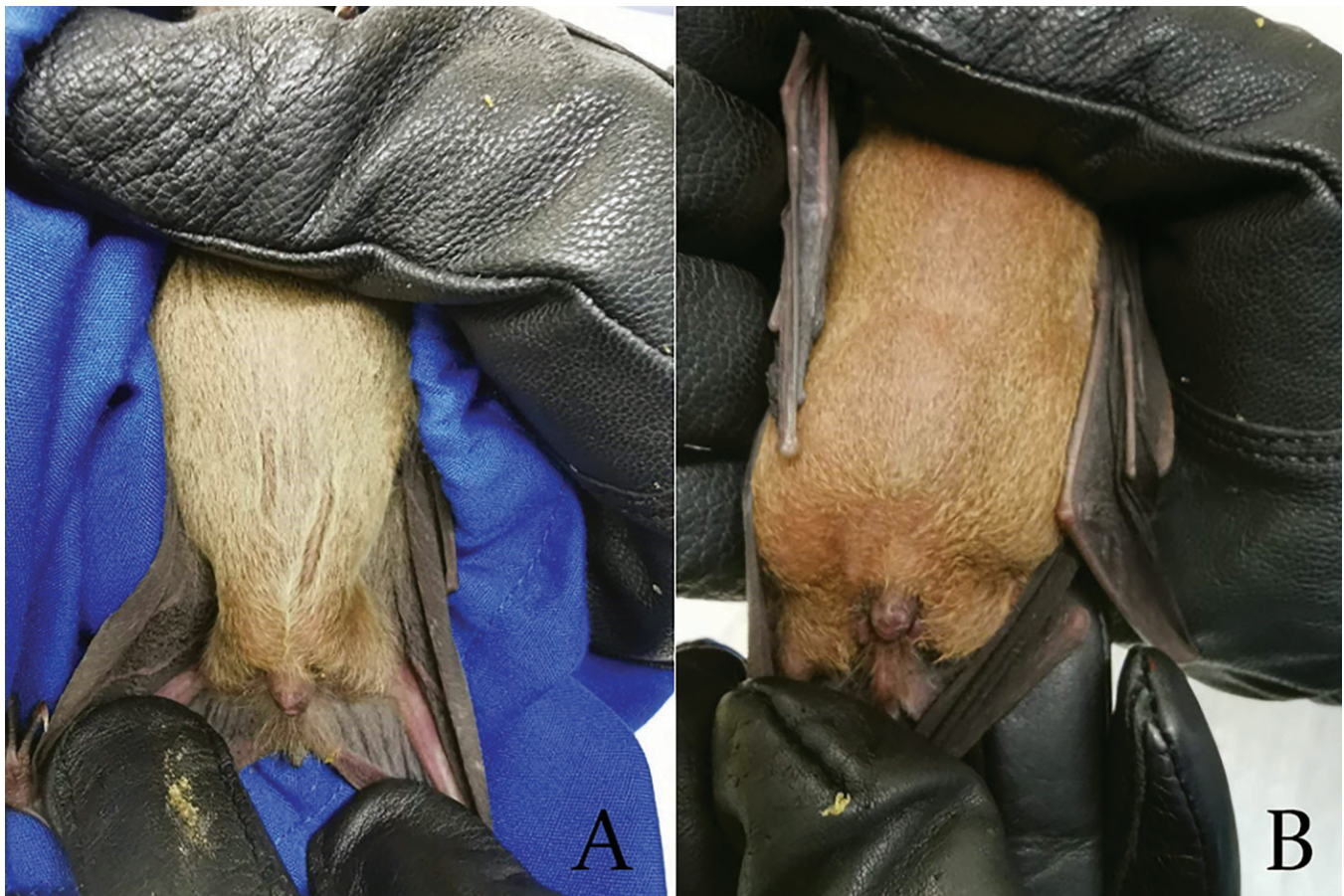


Figure 2. Size of lower abdomen in a) normal and b) gravid bats. Visible distention around the lower abdomen can be observed in gravid bats.

Bats were captured using a butterfly catch net and immediately transferred into holding bags and moved to the Biosafety Cabinet (BSC) in the ABSL-1 laboratory. Bats that appeared weak, emaciated, lost more than 20% of previously measured body weight, or had a poor prognosis were euthanized. For euthanasia, bats were anesthetized in the BSC with isoflurane gas and once anesthetized, were given an overdose of Sodium

Pentobarbital 90 to 100 mg/kg body weight via the intramuscular or intraperitoneal route. This procedure was discussed and selected by the facility veterinarian and research personnel prior to starting the colony.

Sample collection. External (head and body) and internal (oral and rectal) swabs were collected. External swabs were collected by swabbing the head and body of the bats in an

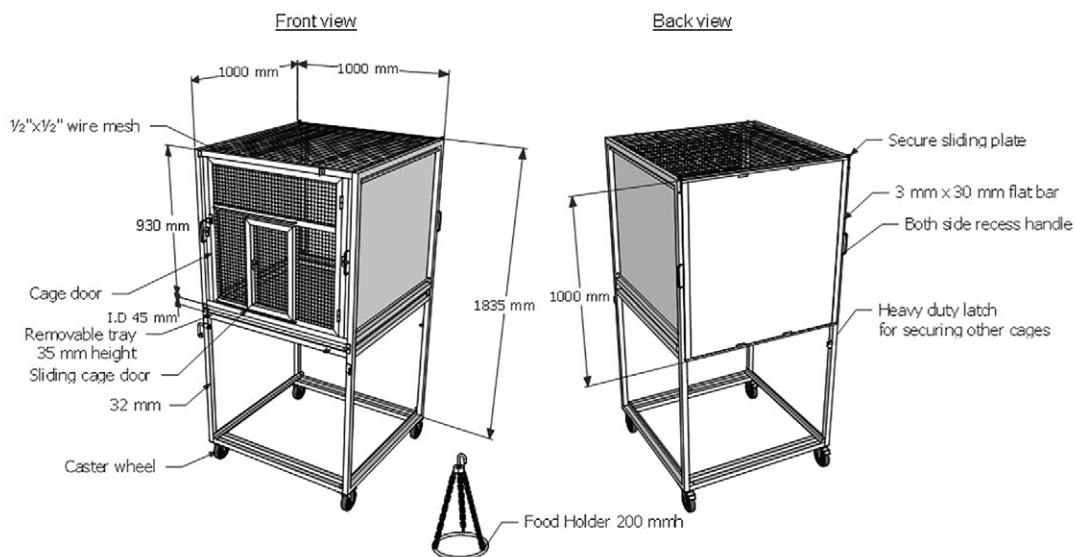


Figure 3. Schematic representation of the bat cage. The bat cage is specifically designed, and custom made to accommodate living conditions of *E. spelaea*. The cage is designed to allow combination of 2 or 4 cages and castor wheels with brakes were also included. Frame, panels, and food holder were made of SUS 304 stainless steel.



Figure 4. Drape hanging over top corner of bat cage to provide more shade and seclusion.

up and down motion with phosphate buffered saline (PBS) (Hyclone, Logan, UT) soaked sterile polyester tipped swabs (Puritan Medical Products, Maine, United States of America). Oral swabs were collected by swabbing the inner cheeks, tongue, and palate. Rectal swabs were obtained by inserting a PBS-soaked sterile polyester tipped swab into the rectum, rotating gently, and removing. All swabs were stored in 2-mL screw cap microtubes (Sarstedt, Nümbrecht, Germany) with 500 μ L of viral transport media (VTM) made up with 10% Bovine Serum Albumin (Sigma-Aldrich, St. Louis, MO) and 20% Antibiotics-Antimycotic (ThermoFisher Scientific, Waltham, MA) in sterile PBS. The samples were stored at -80°C (-112°F). Blood samples were collected by puncturing the cephalic vein of the wing after wiping the puncture site with an alcohol swab. Blood emerging from site of puncture was aspirated with a pipette, suspended in 10 μ L (8 mm) of EDTA, and stored at -20°C (-4°F). Pressure was applied to site of puncture with sterile gauze to stop the bleeding, followed by application of antiseptic cream to prevent infection. Samples were collected from each bat prior to introduction into the colony and during a health check every 3 to 4 mo. After the samples were collected, bats were given 1 mL of fruit juice before release back into their cage. Samples were not taken from bats that were pregnant or nursing. All personnel wore personal protective equipment (PPE), bouffant cap, mask, face shield, and shoe cover when performing sampling.

Measurements. Morphometric measurements (weight, forearm length, and body length) were recorded from each bat prior to introduction into the colony and during medical checks every 3 to 4 mo using a vernier caliper (Coolant-Proof Digimatic Caliper, Mitutoyo, Kawasaki, Kanagawa, Japan).

Table 1. Bat colony daily dietary amounts of optimized diet and supplement. Dietary amount listed below are for 20–25 bats

Diet components	Formulation
Liquid diet Equation (500 mL total volume)	250 mL water 36 g low fat milk powder 0.25 g powdered pollen Top up to 500 mL with warm water
Commercial diet (500 mL total volume)	150 g Wombaroo diet 1 g powdered pollen Top up to 500 mL with warm water
Supplement	10 slices of watermelon / papaya / mango (To sprinkle some fruits with approximately 10 g of milk powder)
Water	250 mL
Salt water	250 mL of water 5 g table salt

Forearm length was measured from the ulna to the wrist (Figure 5a). The total body length was taken by extending the body of the bat and measuring it from the top of the head to the end of the tail (Figure 5b).

Microchipping. Bats were microchipped using ID-100B (1.4) Mini Transponder in Canula (Trovan, Isle of Man, United Kingdom). The back of the neck was cleaned with an alcohol swab and the microchip was injected subcutaneously. Pressure was applied to stop any bleeding, followed by application of antiseptic cream to prevent infections.

Extractions, cDNA Synthesis, and PCR

RNA Extraction. RNA was extracted from whole blood, oral, rectal, body, and head swab samples using an RNA Blood Mini Kit (QIAGEN, Hilden, NRW, Germany) and Qiagen RNeasy Mini Kit (QIAGEN) according to manufacturer's instructions. RNA was eluted in RNase-free water and stored at -80°C (-112°F). RNA concentration and quality were measured using the DeNovix DS-11+ Spectrophotometer.

cDNA Synthesis. cDNA was generated from 1 μ g of RNA using QuantiTect Reverse Transcription kit (QIAGEN) according to manufacturer's instructions and stored at -20°C (-4°F).

PCR Screening. PCRs were performed to detect Filovirus, Coronavirus, Nipah virus, and Rhabdovirus using GoTaq Green Master Mix (Promega, Madison, WI) in a 15 μ L reaction with 40 ng of cDNA.^{4,8,21} Primers and annealing temperature can be found in (Table 2).

Results

Introduction of founding population. In October 2015, 3 female bats were captured and introduced into the facility as a pilot run. After 6 mo of successful integration, 12 female and 4 male bats were captured in April 2016. Wild caught *E. spelaea* weight ranged from 25 to 65 g. Forearm length ranged from 56 to 73 mm (Table 3). Wild caught bats were PCR negative for rabies virus, coronaviruses, filoviruses, and Nipah virus.

Morphometric monitoring of integrated bats. To track the bats' growth and adaptation to the artificial housing, their baseline weight was measured before introduction and at health checks every 3 to 4 mo. At the first health check, 3 mo after introduction to the colony, 16 out of the 19 founding bats showed a weight gain of 1 to 11 g, whereas 3 bats had a weight loss of 1 to 5 g. For female bats, a net gain in weight was recorded 9 to 15 mo after

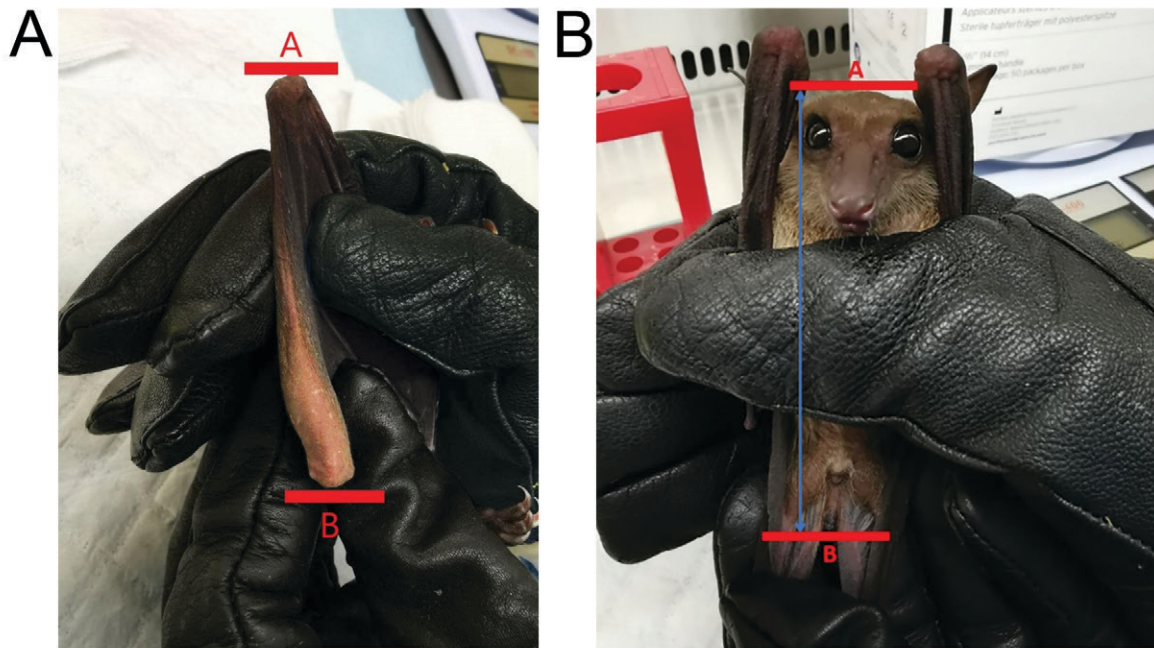


Figure 5. Length of a) forearm and b) body. Forearm length is determined by measuring the ulna from point A to B. Body length is determined by measuring from the point A (top of the head) to point B (tip of the tail).

introduction into the colony before pregnancy or pups latching on (Figure 6) were observed. After 24 mo, male bats showed a net gain in weight. Bats appeared to be alert, and responsive during physical handling and showed no signs of stress, injury, loss of fur, or weakness.

Pregnancy. The gestation period of *E. spelaea* bat is 3 to 4 mo.³ No pregnancy was detected in female bats during the first 6 mo after introduction. An observable enlarged abdomen was the primary indicator of pregnancy in the colony followed by a gain of weight. ES 16 was suspected to be pregnant due to an observable enlarged abdomen during the 9 mo after introduction health check in January 2017. From October 2016 to January 2017, ES 16 gained 19.5 g followed by a 2.5 g decrease in weight in May 2017. In May 2017, one pup without a microchip was found that potentially belonged to ES 16. Pups were also found latching on ES 1, 9, and 19 during the health check in May 2017; females were weighed with the pups attached, which accounted for at least some of the weight gain in females. Bats ES 1, 9,

and 19 were not suspected to be pregnant due to the absence of an enlarged abdomen during the January 2017 health check. Multiple pregnancies and pups have been detected (Table 4) since January 2017. *E. spelaea* are documented to be polyestrous and can give birth to 2 pups a year.¹² However, ES 1 produced 3 pups within a year (January 2018 to December 2018). Pups were found to be latching on ES 1 on April, July and December 2018. (Table 5). Changes in behavior were observed during the early and middle stages of pregnancy. Pregnant bats were generally more aggressive and vocal during handling, whereas bats closer to parturition were more docile and had a larger bulge on the abdomen. Pregnant and nursing bats were returned to the colony immediately after weighing and feeding. A total of 35 bats were born and implanted with microchips between May 2017 and December 2018.

Morphometric tracking of pups born in the colony. Colony-born bats were first identified as bats without a microchip. Nine of 35 pups born on different days were chosen for the

Table 2. PCR primers for Filovirus L gene, Coronavirus RdRp region, Nipah Virus N gene, Rhabdovirus L gene and their expected amplicon size^{4,8,21}

Target	Primer	Sequence (5' to 3')	Annealing temperature	Size (base pair)
Filovirus L gene	FV-F1	GCMTTYCCCIAGYAAYATGATGG		460 bp
	FV-R1	GTDATRCAYTGRTRTCHCCCAT		
	FV-F2	TDCAYCARGCITCDTGGCAYC	54 °C	350 bp
	FV-R2	GIGCACADGADATRCWIGTCC		
Coronavirus RdRp region	IN-6	GGTTGGGACTATCCTAAGTGTA	56 °C	440 bp
	IN-7	CCATCATCAGATAGAATCATCATA		
Nipah N gene	Forward	ATGCCATGGATCCCGAATGAGTGATAT TTTT-GAAGAGGCGGCTAG	51 °C	1500 bp
	Reverse	CGTAGCACTCGAGGATTACACATCAG CTCT-GACAAAGTCAAG		
Rhabdovirus L gene	Forward	CCADMCBTTTGYCKYARRCCTTC		
	Reverse	RAAGGYAGRITTTTTYKCDYTRATG		
	Forward 2	ATGACAGACAACCTGAACAA	58 °C	340 bp
	Reverse 2	CCATTCCAGCAGGTTGGACC		

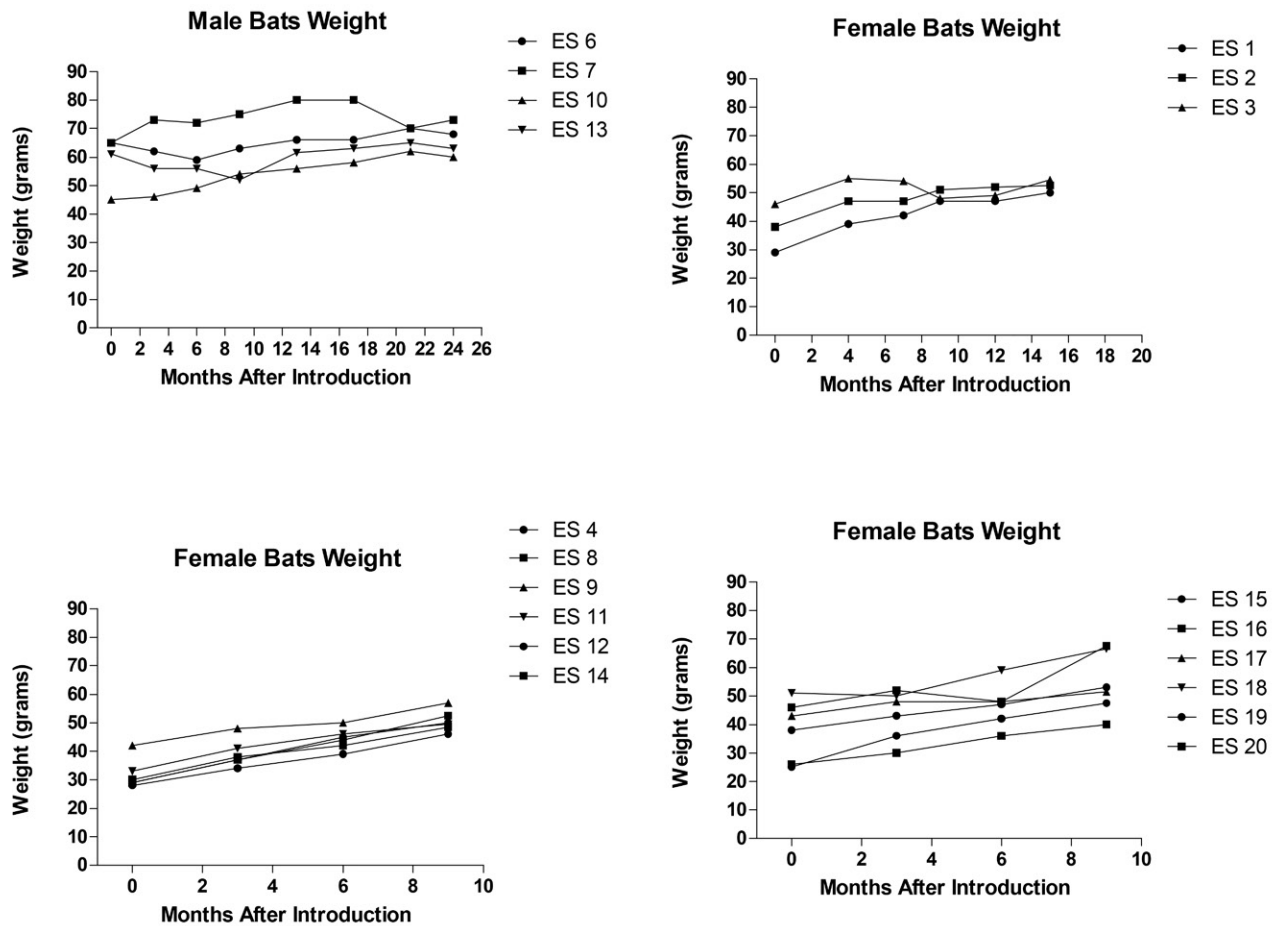


Figure 6. Weight trend of bats in captivity. Increase in body weight of female bats across 9–15 mo and male bats across 24 mo.

morphometric study (Table 6). At approximately 3 to 4 mo old, their weights ranged from 31 to 43 g, total lengths ranged from 78 to 95 mm, and forearm lengths ranged from 55 to

66 mm (Table 6). After 7 to 15 mo in the colony, all pups had experienced a 12 to 27 g increase in weight (Table 6). Forearm length increased by 0.9 mm to 11.2 mm (Table 6). Female *E. spelaea* were fertile from 6 mo old.^{9,18} Of the 2 female pups, ES 37 was suspected to be pregnant 15 mo after birth due to the presence of an enlarged abdomen and weight of 66 g. All pups observed after health checks were bright, alert, and responsive after release into the cage.

Diet. The first diet used was adapted from a previous description.¹⁴ That formulation featured a blend of 2 mashed bananas, 500 mL apple juice, 20 g sucrose, 15 g glucose, 54 g infant formula (Abbott, Singapore), and 500 mL water. To complement this diet, store bought pollen (HoneyWorld, Singapore) can be incorporated into the blend. Each bat is estimated to feed on 20 mL of this diet per day.¹³ In the initial stage for the captive colony, a combination of a liquid diet consisting of apple juice blended with glucose powder, white sugar, and milk powder was given from Oct 2015 to June 2016. Water was given separately in another feeding bowl. The volume of this concoction was provided in surplus to be sure the bats had enough food. This formulation was palatable to bats as little leftover was recorded (Table 7). However, apple juice caused the milk to solidify when mixed, and sediments formed on the bottom of the feed pan. This can potentially cause impaired protein intake from milk powder. After a May 2016 consultation with Tolga Bat Hospital, the diet was further optimized by including Wombaroo Lorikeet and Honeyeater Food and salt water prepared inhouse and removing white sugar and apple juice (Table 1). Although the original diet provided the bats with enough sugar, bats require minerals including sodium, which are lacking in fruits.^{10,11} The

Table 3. Wild *Eonycteris* values for introduction date, sex, and morphometric measurements

<i>Eonycteris spelaea</i> (ES#)	Date Introduced (dd-mm-yy)	Sex	Weight (grams)	Forearm (mm)
6	13-04-16	Male	65	71.5
7	13-04-16	Male	65	73.4
10	14-04-16	Male	45	66.9
13	27-04-16	Male	61	68.1
1	22-10-15	Female	29	59.7
2	22-10-15	Female	38	63.5
3	22-10-15	Female	46	65.6
4	13-04-16	Female	28	58.1
8	13-04-16	Female	29	58.4
9	14-04-16	Female	42	64.7
11	14-04-16	Female	33	65.8
12	27-04-16	Female	29	59.7
14	27-04-16	Female	30	59.8
15	27-04-16	Female	25	59.5
16	27-04-16	Female	46	65.5
17	27-04-16	Female	43	68.5
18	27-04-16	Female	51	68.4
19	27-04-16	Female	38	62.5
20	27-04-16	Female	26	55.9

Table 4. Female *Eonycteris spelaea* pregnancy tracking, abdomen enlargement and weight measurement up to 23 mo in captivity

<i>Eonycteris spelaea</i> (ES#)	Months in captivity	Health check date	Weight (grams)	Enlarged abdomen	Status
1	12	October 2016	47	No	Normal
	15	January 2017	50	No	Normal
	19	May 2017	80	–	With pup
	23	September 2017	66	No	Normal
	2	12	October 2016	52	No
2	15	January 2017	52.5	No	Normal
	19	May 2017	54	Yes	Pregnant
	23	September 2017	52	No	Normal
	3	12	October 2016	49	No
3	15	January 2017	54.5	No	Normal
	19	May 2017	54	Yes	Pregnant
	23	September 2017	75	–	With pup
	4	6	October 2016	39	No
4	9	January 2017	46	No	Normal
	13	May 2017	57	Yes	Pregnant
	17	September 2017	49	No	Normal
	8	6	October 2016	44	No
8	9	January 2017	52.5	No	Normal
	13	May 2017	58	No	Normal
	17	September 2017	70	–	With pup
	9	6	October 2016	50	No
9	9	January 2017	57	No	Normal
	13	May 2017	84	–	With pup
	17	September 2017	74	–	With pup
	11	6	October 2016	46	No
11	9	January 2017	49.5	No	Normal
	13	May 2017	51	No	Normal
	17	September 2017	72	–	With pup
	12	6	October 2016	45	No
12	9	January 2017	50	No	Normal
	13	May 2017	53	No	Normal
	17	September 2017	53	No	Normal
	14	6	October 2016	42	No
14	9	January 2017	48.5	No	Normal
	13	May 2017	52	No	Normal
	17	September 2017	57	No	Normal
	15	6	October 2016	42	No
15	9	January 2017	47.5	No	Normal
	13	May 2017	51	No	Normal
	17	September 2017	72	–	With pup
	16	6	October 2016	48	No
16	9	January 2017	67.5	Yes	Pregnant
	13	May 2017	65	No	Normal
	17	September 2017	58	No	Normal
	17	6	October 2016	48	No
17	9	January 2017	51.5	No	Normal
	13	May 2017	63	No	Normal
	17	September 2017	58	No	Normal
	18	6	October 2016	59	No
18	9	January 2017	66.5	No	Normal
	13	May 2017	76	Yes	Pregnant
	17	September 2017	65	No	Normal

Table 4. (Continued)

<i>Eonycteris spelaea</i> (ES#)	Months in captivity	Health check date	Weight (grams)	Enlarged abdomen	Status
19	6	October 2016	47	No	Normal
	9	January 2017	53	No	Normal
	13	May 2017	84	–	With pup
	17	September 2017	78	–	With pup
20	6	October 2016	36	No	Normal
	9	January 2017	40	No	Normal
	13	May 2017	45	No	Normal
	17	September 2017	45	No	Normal

Wombaroo diet contains 12% protein, 5% fat, 76% carbohydrate, 4% ash, 3% moisture, and 17 MJ/kg energy.²⁷ The final formulation provided carbohydrates, protein, fat, essential amino acids, vitamins, and salt. After introduction of the new formulation, the food provided was consumed with little leftover. (Table 7).

Discussion

We identified a number of factors that are essential in establishing an *E. spelaea* breeding colony. The first challenge was to create housing conditions that allowed the bats to breed, roost, and feed. A breeding colony of *Carollia perspicillata* in Germany used cages 81 cm in long, 170 cm wide, and 77 cm high. Cages were kept in rooms with regulated light cycles. During the light phase, black plastic sheets were hung on top of cages to provide shade and concealment in the cage.²³ In comparison, our housing featured 2 cages with combined measurements of 200 cm long, 100 cm wide, and 76 cm high, providing ample space for bats to fly. Hessian fabric retains the scent of the bats, providing an additional sense of security and familiarity. Our cage design allows cages to be linked together to accommodate colony expansion. No injuries were seen during health checks, indicating that the cage design is not detrimental to the safety of juvenile and adult bats.

The artificial environment (cages) must be coupled with a suitable diet. Therefore, the diet was optimized to provide adequate nutrients for the bats, as a commercially available feed is not available for nectarivorous bats. The optimized diet included Wombaroo diet, which contains the required nutrients for all nectar-eating birds. The Wombaroo diet was designed to recreate the texture and nutritional contents of nectar and provide sufficient protein like pollen.²⁷

After introduction, bats were monitored for abnormal behavior such as self-isolation and weight loss, which is often an

Table 5. Female *Eonycteris spelaea* #ES1. This animal had 3 pregnancies and 3 pups in a span of 1 y *Weight was not taken during June 2018 check

Months in captivity	Date	<i>Eonycteris spelaea</i> (ES#1)	
		Weight (g)	Status
26	January 2018	64.0 g	Pregnant
29	April 2018	88.0 g	With pup
30	June 2018*	—	Pregnant
31	July 2018	75.0 g	With pup
35	October 2018	66.0 g	Pregnant
37	December 2018	69.0 g	With pup

Table 6. Weight and forearm length gains of *Eonycteris spelaea* pups at 3 to 4 mo of age and again at 7 to 15 mo with difference between the 2 tests

ES#	Sex	Weight (g) at 3–4 mo of age (g)	Weight (g) at 15 mo of age	Weight difference (g) between 15 and 3–4 mo of age	Forearm length (mm) at 3–4 mo of age	Length (mm) at 7 to 15 mo of age	Forearm length differences (mm)	
							Forearm length (mm) between 15 and 3 to 4 mo of age	between 7 to 15 and 3 to 4 mo of age
5	Male	36	53	17	57.3	66.7	57.3 (15 mo)	9.4
37	Female	43	55	12	61.3	65.2	61.3 (7 mo)	3.9
38	Male	35	62	27	58.9	65.9	58.9 (11 mo)	7
39	Male	42	60	18	66	66.9	66.0 (11 mo)	0.90
40	Male	40	62	22	61.4	67.5	61.4 (11 mo)	6.1
52	Male	42	63	21	63.4	69.5	63.4 (11 mo)	6.1
53	Female	38	54.8	16.8	60.4	67.2	60.4 (11 mo)	6.8
54	Female	31	51	20	55.8	67	55.8 (11 mo)	11.2
55	Male	43	66.5	23.5	62.1	70.3	62.1 (11 mo)	8.2

indication of stress or illness. Bats ES 6, 13, and 18 lost weight, potentially due to problems acclimatizing to the diet and housing. Over 9 to 24 mo, these 3 bats gained weight, suggesting that they had successfully adapted to the colony. Generally, fruit eating bats are bimodally polyestrous, generating 2 offspring per year.²² Our *E. spelaea* colony showed signs of pregnancy after 9 to 15 mo in the colony. Similarly, in the *C. perspicillata* breeding colony, breeding started after 11 to 12 mo.²³ ES 1 produced 3 offspring from 3 separate birthing events between January and December 2018, potentially implying that given proper housing conditions and ample food, breeding can be aseasonal. Also, given an abundance of resources and the lack of natural threats, the number of pups an *E. spelaea* female could produce in any given year is solely restricted by the bat's ovulation cycle. Another plausible explanation was that ES 1 had undergone superfetation which means a second conception occurred in a pregnant mammal.³ Bats are known to have developed multiple reproductive strategies such as sperm storage, delayed fertilization, and delayed implantation.^{17,22} More in-depth studies will need to be conducted to investigate superfetation and possible delayed reproduction in the colony.

A healthy fur coat and responsiveness were used as markers of general good health of the bats. No signs of injury, joint inflammation, or drastic loss of weight were seen. In the 16 mo period from introduction of the wild bats into the colony to the birth of new pups, no sign of trauma was observed in adult bats or pups. Taken together, the artificial housing and diet created a conducive environment for the bats to thrive.

Table 7. *Eonycteris spelaea* diet given over amount eaten over a 3-mo average after optimization

Substance	Diet intake during a 3-mo period before Diet Optimization		Substance	Diet intake during a 3-mo period after Diet Optimization	
	Amount Provided	Amount Consumed		Amount Provided	Amount Consumed
Liquid diet	250 mL	247 mL	Liquid diet	500 mL	250 mL
Fruit slices	4	4	Wombaroo diet	500 mL	500 mL
Water	250 mL	122 mL	Fruit slices	10	10
			Salt water	250 mL	62 mL
			Water	750 mL	379 mL

The study of bats and their responses to infection is essential in understanding the transmission and infection dynamics of viruses. Despite experiencing viral infections, bats generally do not display any clinical signs of disease. Learning more about the mechanisms used by the bat immune system to coexist with or evade viruses is relevant to both human and animal health.¹ The immunologic profile of bats varies during migration seasons and among species.^{2,24} Previous and ongoing studies on bats have relied on wild caught bats whose health and immune status varies among the sampled bats.¹⁵ Thus, the results of these studies may be skewed because the baseline is unclear. Access to a breeding colony mitigates some of these challenges. Furthermore, the study of bat-virus interaction is presently hindered the lack of species-appropriate molecular biology reagents, indicating the urgent need to allocate resources and collaborative efforts to further investigate the synergy and relationship between bats and bat-borne viruses.² While hurdles still exist in bat research, this breeding colony represents a step forward in understanding the bat immune system. This study concludes the successful establishment of the first *E. spelaea* breeding colony in Asia. Bats produced in the colony and their samples are currently being used in ongoing studies.^{19,20,26,28}

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Author contributions

L.-F.W., J.H.J.N. and Y.T.C. conceptualized and designed the study. R.F. and Y.Y.H. prepared the manuscript. L.-F.W., J.H.J.N., I.H.M., Y.T.C. and W.N.C. revised the manuscript. Y.T.C., R.F., Y.Y.H., Lee P.Y.-H., I.H.M., D.L.H.W., S.A.B., W.N.C., K.P.S., R.F. and Ng J.H.J. caught wild bats. J.H.J.N., Y.T.C., R.F., Y.Y.H., W.N.C., A.E.Z.K. and K.P.S. performed

health screens and experiments. E.M.P and R.Y. perform health screens and husbandry. N.B.S. manages the facility operations.

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