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Posted Date: 12 March 2026

doi: 10.20944/preprints202603.0997.v1

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Household Bat Guano Farming in Rural Cambodia: Farming Practices, Viruses, Spillover Risks, and Recommended Mitigation Measures in Guano-Producing and Neighbor Households

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Abstract

In Cambodia, farmers construct artificial household bat roosts to collect and sell guano as fertilizer. We investigated farming practices and attendant spillover risks using: 1) surveys on guano production; 2) estimating bat population size and species present using carcasses, visual identification, and audio recordings; 3) surveying guano-producing and neighbor households on water, sanitation, and hygiene practices; and 4) testing guano and household food, water and surfaces for coronaviruses by PCR. Bat roosts are constructed using dried palm leaves with coconut tree and/or steel/concrete supports. Roosting areas ranged from 42-327 m^2, bat abundance varied from 0-11,187, guano production was 5-120 kg/week, guano yields were 0.15-0.4 kg/m2/week, and farmers earned ~100-200 USD/household/month. Higher guano production in peak (normally wet) season was associated with greater bat abundance ($p=0.016$). The lesser Asiatic yellow house bat (*Scotophilus kuhlii*) was the only bat species identified. Roosts were <20 m from guano-producing households. Neighbors and households' hygiene risks included not having handwashing stations and not covering food in storage/while drying. Alphacoronaviruses or Infectious Bronchitis Virus were found in 14.6%, 17.3%, 2.9%, 1.4%, and 0.0% of guano, urine, surface, food, and water samples, respectively. While guano farming offers economic benefits, spillover risks exist. Safe guano collection and storage, handwashing, and food covering in guano-producing communities are necessary to mitigate spillover risks.

Keywords: bat roost; coronaviruses; guano; surface contamination; viral spillover

 doi:10.20944/preprints202603.0997.v1

1. Introduction

Bat guano is commonly used as an organic fertilizer, rich in carbon, nitrogen, minerals, and microbes [1,2]. Valued at anywhere between $1.25 to $12 per pound [2,3], bat guano is found in and harvested from caves [1,4,5]. This is common in Cambodia, where 37 of 74 known bat species are found in caves [4,6], and bat guano harvesting has been evident in at least 38 known caves [4]. Beyond caves, in the Mekong Delta region of Cambodia and Vietnam, farmers construct artificial roosts to attract free-ranging bats and collect guano [7]. These artificial roosts, often made of locally sourced materials such as palm fronds and supported by coconut trees or poles, are a common source of income for farming households. However, detailed studies on the specific methods, bat species involved, and associated zoonotic risks in these systems remain limited.

Zoonotic disease risks are inherent in human-bat interactions during guano harvesting [7]. In commercial harvesting from caves, guano harvesters may be exposed to certain hazards, including animal bites, dust inhalation, and infectious diseases [8]. This is evident in Uganda, where bat exposure among those living near bat roosts was positively associated with being male, living in urban settings, hunting, and perceiving guano as a safe fertilizer [9]. These risks are exacerbated by poor knowledge among guano farmers. In Uganda, about 43% of participants thought guano was a safe fertilizer option [9]. Among guano miners in Thailand, 36% reported doing nothing or not knowing what to do in the event a bat bit them [10]. In formative research for this study, bat guano farmers in Cambodia were found to have limited awareness of zoonotic risks and employed suboptimal biosafety measures, such as handling guano without personal protective equipment (PPE), and a lack of adequate water treatment and hygiene practices (unpublished data). These findings are consistent with past research that documents close interactions between communities and cave-roosting bats in Northwest Cambodia [11]. Thus, there is a need to understand the spillover risks of having bats close to households and communities.

Cambodia is home to at least 80 bat species [12], including *Mops plicatus, Taphozous* spp., *Hipposideros larvatus, Rhinolophus microglobosus*, *H. armiger*, and *R. shameli* [4]. Bats are reservoirs for viruses such as coronaviruses, Nipah virus, and astrovirus, which have the potential to spill over into human populations [13]. Nipah virus infection has been detected Cambodian bat populations, though no evidence of human cases has been recorded [14]. Coronaviruses are also common findings in Cambodian bats, with positive polymerase chain reaction (PCR) detection in 4.2% of cave-dwelling bats in Kampot Province and 4.75% flying-foxes in Kandal Province in Cambodia [15]. Coronavirus shedding was most frequent among juvenile and immature Cambodian bats [15]. The transmission of RNA viruses, particularly coronaviruses, can occur between bat species, which creates further opportunities for viral evolution and spillover [16]. There is a deep reservoir of coronavirus diversity in Southeast Asian bats, much of it undescribed, and one of the closest known relatives of SARS-CoV-2 was detected in *R. shameli*, known as the horseshoe bat, from northern Cambodia [17].

Proximity between artificial bat roosts and households represents a risk factor for zoonotic spillover. Zoonotic pathogens could spread through direct contact with guano during harvesting, airborne exposure to particulates, or contamination of household food and water supplies [13]. Epidemics of coronaviruses such as SARS-CoV and Swine Acute Diarrheal Syndrome-Coronavirus have been attributed to bats in Southeast Asia [18]. Sánchez *et al.* (2022) estimate that as many as 66,000 are infected with SARS-related coronaviruses annually in South Asia [13]. Living close to bat roosts puts farmers and their families, communities, and livestock at risk of disease. Beyond the impact on human health and spillover risk, there are also implications for economic development and health system capacity.

Despite advances in understanding zoonotic disease risks, significant gaps remain. While previous studies have identified a variety of viruses in free-ranging bat populations, the mechanisms of spillover and human infection pathways are not fully understood [19]. Additionally, sociocultural factors influencing guano harvesting practices require further exploration to develop culturally appropriate interventions [11]. To fill these research gaps, we conducted four related activities to understand guano farming practices and spillover risks: 1) surveys with guano farmers focused on

 doi:10.20944/preprints202603.0997.v1

production and farming; 2) estimates of bat population size and determination of species using biometrics and visual and audio recordings; 3) surveys of guano-producing and neighbor households on water, sanitation, and hygiene (WASH) practices; and 4) testing of guano, urine, and household food, water, and surfaces for coronavirus RNA. The goal of our research was to describe farming practices and identify spillover risks, to enable development of interventions to reduce spillover risks as part of the larger STOP Spillover project conducted by Tufts University and partners.

2. Materials and Methods

This study was conducted in three communities located in the central lowlands of Cambodia in Kampong Cham province. Kampong Cham has a population of 895,763 people [20], where most (51-53%) income is derived from agriculture [21]. This area was selected for study inclusion because it had been identified in previous STOP Spillover work as having households engaged in guano farming. Working with community leaders and local authorities, we identified 17 guano farms in these three communities for study inclusion.

To answer the research questions, data were collected during farming practice surveys and observations with farmers, overnight observations and sample collection on guano farms, and household WASH surveys and sample collection with guano-producing and neighbor households. The framing practices survey questionnaire was designed to collect data on guano farming practices, including demographics, farm size, roost construction materials and style, roost management practices, bat species occurrence, abundance, and seasonality, historical and current guano production and sales, and beliefs about factors affecting bat populations. Lastly, bat roost structures were photographed, sketched, and measured (height to lowest leaves, length, width) using a laser rangefinder (Leica, Wetzlar, Hesse, Germany). Trained and supervised enumerators conducted these approximately 30-minute surveys with all 17 identified households. Survey data were collected using KoboToolbox (Cambridge, MA, USA) on a Samsung (Suwon, South Korea) tablet, with paper forms available for backup. Data was exported into Microsoft Excel (Redmond, WA, USA) for cleaning, and descriptive analyses. Statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria). Phylogenetic tree analysis was fitted with Muscle 2.1 in Geneious Prime (Biomatters Ltd.) and generated via Neighbor-joining and Tamura-Nei genetic distance model. The outgroup was set as SARS CoV2 human USA

To estimate bat abundance, a wired digital video recorder (DVR) camera system (Zosi Technology, Hong Kong SAR, PRC) was installed for one overnight observation per farm. This system included four infrared-enhanced video cameras, a DVR recorder, a 12V lead-acid battery, and a voltage regulator. System set-up was completed before the evening emergence period, when bats exit roosts in search of food [22]. To avoid double-counting and investigate the peak emergence period, counts were made for one hour from first observed bat emergence. The first and last bat departure times (and any returns) for each camera within the first hour were also noted. Lastly, images and video of the entire guano farm were used to estimate the proportion of the perimeter covered by the camera system. In data analysis, this proportion was used to correct observed bat numbers by dividing the count by the estimated proportion under observation. To estimate bat species, data were collected concurrent with survey visits and with bat abundance estimation. Data from these investigations were triangulated to compare results and identify bat species present.

During farmer survey visits, opportunistic searches were undertaken for bat carcasses underneath roost structures. If found, these were photographed, and their forearm lengths measured with a digital vernier caliper from the extremity of the elbow to extremity of the carpus with the wings folded. Species were identified in accordance with standard morphological criteria [23,24]. To obtain an additional data point and better understand farmers' knowledge, a placard with 12 unlabeled images of 11 bat species was presented (Figure A1). Each respondent was asked to select species they believed present on their farms.

During overnight observation, acoustic data were collected at each farm using Song Meter 4 full-spectrum bat detectors fitted with calibrated U2 ultrasonic microphones (Wildlife Acoustics,

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Maynard, MA, USA). A single device was deployed for one night at each farm and programmed to record from 30 minutes before sunset until sunrise. Acoustic data were analyzed through visual inspection of recordings (via call frequencies, structure, and duration) in Adobe Audition (Adobe Systems, San Jose, CA, USA) and Batsound (Pettersson Elecktronic, Uppsala, Sweden). Identifications were made to the lowest taxonomic level possible based on datasets of verified recordings for known bat species from Cambodia (Neil Furey, personal correspondence).

Deviations from normal distribution were tested using the Shapiro-Wilk Test. Estimated bat abundance was log-transformed when assumption of normality was violated. Correlation of pairs of normally distributed variables were assessed using linear regression. Differences in proportions between groups were assessed using Fisher's Exact Test. Statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria).

For bat guano collection, before dawn in the overnight observations, a 1 m^2 plastic sheet for every 10 m^2 of occupied roosting area was placed under roosts to non-invasively collect guano and urine samples. Two guano samples and one urine sample per sheet were collected. After 15 minutes, fecal samples were scooped up using plastic spoon-tip straws, and urine was collected by absorption onto sterile polyester swabs (Copan Diagnostics, Murrieta, CA, USA). Both sample types were then placed into a 2 mL cryovial (CryoKING, Biologix Group LTD, Changzhou, China), containing 0.9 mL of DNA/RNAShield (Zymo Research, Irvine, CA, USA) and kept on ice until reaching the laboratory, where they were kept at -20°C until testing.

Regarding food, water, and surface survey and sample collection, data were collected from 10 randomly selected guano farms and the 10 nearest adjacent neighbor households. The number of households sampled was determined based on laboratory capacity, funding and time availability, and the expected number of households required to document variation in household practices. Enumerators received training on informed consent, personal protection, survey implementation, and standard operating procedures for surface, food, and water sampling. Concurrently, dedicated enumerators conducted approximately 60-minute interviews with households related to food, water, and surface practices while other dedicated enumerators wrote down a map of the households and selected approximately 10 samples from surfaces and food, and water samples of drinking and irrigation water. After surfaces were selected, an alcohol- and bleach-disinfected 10x10 cm stencil was placed on the surface, and a sterile polyester swab was swiped across the surface in horizontal, vertical, and diagonal directions. The swab was then placed in a tube prefilled with 0.9mL of DNA/RNAShield. The tube was sealed and transported to the laboratory in a cooler with ice. Samples were stored at the laboratory at -20°C until analysis. Duplicate water samples of 500 mL were collected from drinking water and irrigation water storage containers into Whirl-Pak® bags (Pleasant Prairie, Wisconsin, USA) and stored on ice until reaching the laboratory.

All guano, urine, surface, food, and water samples were tested for presence of CoV RNA at the Institut Pasteur du Cambodge virology laboratory. Water samples were pre-processed by transferring 100 mL into a vacuum-driven filtration system using a Stericup® (Millipore Sigma, St Louis, Missouri, USA). Water flowed through a 0.45µm PVDF membrane filter supported by a filter support. The guano samples were first centrifuged (GT 422 centrifuge, 3,000 rpm, 2 min, 4°C) to remove any sample material from caps, before being homogenized using a pellet pestle. Subsequently, samples were vortexed ~20 seconds and centrifuged. Samples were then pooled, with 5 samples/pool (100µL per individual sample). Pooled samples were then vortexed and centrifuged (3000 rpm, 15 minutes). The supernatant was recovered and filtered using a syringe filter with 0.45µL pore size (Thermo ScientificTM NalgeneTM Sterile Syringe Filters). RNA was extracted from each pool using the Zymo Research Direct-zol RNA MiniPrep kit (Zymo Research, California, USA), and cDNA was transcribed using SuperScript III First-Strand Synthesis Super-Mix (Invitrogen, San Diego, California, USA). Pan-CoV conventional hemi-nested RT-PCR targeting the RdRp gene was performed as previously described [25] in a 50 µL reaction volume.

All positive samples by pan-CoV conventional RT-PCR were subsequently sent for Sanger sequencing at Macrogen, Inc. (Seoul, Republic of Korea). Sequencing was performed in forward and reverse directions using primers from the second round of the hemi-nested PCR. The sequences obtained were confirmed for similarity using the National Center for Biotechnology Information (NCBI) BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Positive pools were disaggregated and samples run via the same protocol to yield individual results. Results of coronavirus testing were entered into Excel. Initial phylogenetic sequence analysis was performed using Geneious Prime 2022.1.1 (Biomatters Ltd, Auckland, New Zealand) and Blast Search (NCBI). The nearest 100 matches were recovered from Genbank (NCBI) and pairwise identity used for comparison.

3. Results

Guano farming surveys and roost measurements were completed at 17 farms, with bat abundance estimates and bat identification completed at 11 of these due to time constraints, equipment malfunctions, and limited funding (Table 1, Figure A2). A total of 480 guano samples (261 feces and 219 urine samples) from 17 guano farms were collected over four seasons, accounting for seasonal variations and bat reproductive phase in pathogen detection. Food, water, and surface samples were collected from 10 guano farm households and 10 neighbor households, and included 491 samples (346 surfaces, 70 food samples, 75 water samples).

Table 1. Sampling framework.

Sample Type	Quantity	Collection Date
Farmer survey	17 farms	April-May 2023
Household and neighbor WASH survey	20 farms	April 2023
Bat abundance	11 farms	April-May 2023 August 2023 December 2023 March 2024
Bat species identification	7 carcasses (6 farms)	April-May 2023
Bat feces samples	261 samples (17 farms)	April-May 2023 August 2023 December 2023 March 2024
Bat urine samples	219 samples (17 farms)	April-May 2023 August 2023 December 2023 March 2024
Household surface samples	346 samples (10 farms)	April 2023
Food samples	70 samples (10 farms)	April 2023
Water samples	75 samples (10 farms)	April 2023

3.1. Farmer Surveys

Among 17 surveyed farms, most respondents (n=16, 94%) had completed primary school, and just over half (n=9, 53%) were women. The average age of respondents was 59.8 years old, and mean household size was 4.7. Most farms (n=16, 94%) were located <20 meters from households, reportedly to discourage theft and predation by snakes or owls. Men (n=29, 49%), women (n=22, 37%), boys (n=5, 8%), and girls (n=3, 5%) reported working farming, with men and women spending <1 hour/day working. Women (n=16, 94%) were more frequently involved in packaging and drying guano, cleaning the farm, clearing away dead bats, and changing the nets to filter guano than men (n=12, 71%). Men (n=17, 100%) and women (n=15, 88%) both participated in guano collection from under the roosts. The only task almost always performed by men (n=16, 94%) was changing bat roost leaf bunches. Seventy-six percent (n=13) of surveys reported that women were responsible for decision-

 doi:10.20944/preprints202603.0997.v1

making on roles and responsibilities at the farm, 41% (n=7) for guano price, and 53% (n=9) for interacting with buyers. Nearly all farmers (n=16, 94%) reported their primary income was guano farming, and almost all (n=16, 94%) reported earning between 101-200 USD/month from farming.

In all farms, roosts were composed of dried sugar palm (Borassus flabellifer) leaf bunches suspended from supports (Figure 1). Six farms had traditional coconut tree roosts (three dome and three linear). Eleven farms had modern (steel/concrete support) roosts. Respondents reported guano farms are only successful in areas with naturally occurring bat populations. Farmers reported originally building roosts to collect bat guano for use as fertilizer in family vegetable gardens. Later, to respond to demand for natural fertilizer, the bat guano businesses were expanded, commercialized and passed from one generation to the next. All 17 (100%) of farms surveyed used plastic netting to collect guano, 11 farms (65%) raised nets above ground level, and six (35%) kept nets at ground level. Respondents reported changing leaf bunches every other year to reduce parasite loads and avoid bats abandoning the roosts.

(**a**) (**b**) (**c**)

Figure 1. Traditional dome roost (a), traditional linear roost (b), and modern roost on artificial supports (c).

Farm roosting areas ranged from 42-327 m2 and production ranged from 5-49 kg/week during the dry season, with peak output 10-120 kg/week during the wet season. Mean yield of guano varied seasonally between 0.15-0.4 kg/week/m2 of roost area. According to the participants, guano typically sells for 1.25 USD/kg. Most producers collect between 2-3 bags/week, weighing 20-22 kilograms/bag, with a total value between 50-75 USD/week.

3.2. Bat Species and Behavior

A total of seven carcasses were found on six of 17 farms (35%). All carcasses were identified as *Scotophilus kuhlii* based on morphology. Acoustic recordings were obtained at 14 farms, and analysis confirmed all but a small minority of signals closely matching those emitted by *S. kuhlii* (Figure A3). Based on the image lineup of bat species (Figure A1) shown to respondents, 12 (80%) identified an image of *S. kuhlii,* while three (20%) selected an image of *Murina walstoni,* a forest-dwelling species not known from this region of Cambodia.

In general, bats began foraging flights around 18:00 (10-20 minutes after sunset) and began returning at 21:00-22:00. There was continuous traffic in and out of roosts during the night. All bats were observed returning by 04:00-05:00. The timing and direction of the emergence flights was accurately predicted by farmers. Farmers reported female bats give birth to up to two pups per year in April/May and believe deaths are caused during pregnancy, when young bats learn to fly, during hot weather, and in heavy rains.

Estimated bat abundance varied between zero (unoccupied sites) to 11,187 individuals, with an average of 3558. Roost area and estimated bat abundance were not significantly associated (p=0.449). Peak bat guano production was strongly associated (p=0.016) with estimated bat abundance.

 doi:10.20944/preprints202603.0997.v1

3.3. Food, Water, and Surface Survey

The survey was conducted with 10 guano farming and 10 neighboring households. Most respondents were female (n=19, 74%), with an average age of 56.7 years. Literacy rates were high, with 80% (n=20) of female and 89% (n=19) of male heads of households able to read, and 80% of all respondents having attended school to at least primary school level (n=20).

In the following results presentation, n=10 for guano farming and non-guano farming households, unless otherwise mentioned. All households had access to wired electricity. House construction varied, with all guano farming and 50% of neighboring households having wooden walls, and 80% of guano farming and 60% of neighboring households having dirt floors. Additionally, 80% of guano farming and 70% of neighboring households had tile roofs.

Household taps were the primary source of drinking water for 80% of guano farming and 50% of neighboring households. Protected wells were used for non-drinking water by 40% of all households. Most respondents (100% of guano farming and 80% of neighboring) reported covering their water storage containers. Water treatment practices varied, with 70% of guano farming households reporting treating their water compared to 50% of neighboring households, primarily through boiling. All households used water for vegetable cultivation, with 70% of guano farming and 40% of neighboring households covering their water storage containers used for irrigation. Overall, 30% of guano farming and 40% of neighboring households used refrigerators for food storage, with additional storage options including bags and cool boxes. Notably, 80% of both guano farming and neighboring households dried food or meat outside without covering it.

Overall, 90% of households had dedicated cooking spaces, and most respondents (90% farming, 70% non-farming) also had designated dishwashing areas. Soap and sponges were commonly used for cleaning dishes, and 70% of all households used wet mops with detergent to clean their floors. Individual household latrines were used by 80% of guano farming and 100% of neighboring households, and all respondents reported using soap to clean latrines and wash hands. However, only 20% of guano farming households had dedicated handwashing stations (with 0% of neighboring households having this), and only 20% of respondents used running water for handwashing. Trash was stored in bags and burned by all households.

All guano farming and 60% of neighboring households were located within 20 meters of bat roosts. Disturbances from bats, such as entering the house, water contamination, bat excreta, and bat noise, were reported by 10% of neighboring households. In total, 346 surface, 70 food, and 75 water samples were collected from the 20 households. Surface samples were collected from bat roosts, baskets over food, ceramic containers for bat guano, clothes (near bat roost, in and out house), cooking tables, covers on rice, hats for collecting bat guano, outside tables, plates (inside in kitchen uncovered and outside kitchen), railings, tables, fridges, tables near stove, toilet doors, upstairs floor, upstairs table, and water containers (outside and in the kitchen). Surface samples from food included from dried bananas, coconut wastes, dried fish, dried pork, green vegetables (leftovers), jackfruits, leftover fish, leftover pork, mangos, oranges, potatoes, raw meat, rice, sugar cane, tomatoes, and vegetable waste or garbage. Water samples were collected from drinking water and irrigation water storage containers.

3.4. Guano, Urine, Food, Water, and Surface Results

A total of 38 fecal samples (14.6%) and 19 urine samples (17.3%) were positive for coronaviruses (Tables A1 and A2). The overall positivity rate of both fecal and urine samples was 15%, but average positivity percentage by farm ranged from 0-50% (with higher percentages in small farms). Sample-level positivity was higher during rainy season (21.7%) compared to dry season (12.4%), and this difference was statistically significant (p=0.030). Significant differences (p=0.01247) in sample-level positivity were also observed based on bat reproductive periods. Samples collected in March at bat prenatal phase had sample-level positivity of 24.3%. This declined to 17.2% in the bat pupping phase (April-May), 5.7% in the juvenile phase (August), and 2.7% in the adult phase (December).

Positive results, either alphacoronaviruses or Infectious Bronchitis Virus (IBV), were detected in 11 (2.9%) of the 376 surface samples. Alphacoronaviruses were detected in seven samples from guano farming households. Six positive samples were from bat roost surfaces collected from five of the 10 (50%) guano farming households, and one sample was an outside table surface collected from one guano farming household (10%). IBV was detected on four household surfaces, including food covers at one guano farming household and a kitchen table, outside table, and upstairs table at neighboring households. IBV was detected on one (1.4%) of 70 food samples, specifically from a coconut surface collected at a guano farming household. No viruses were detected in the 75 water samples. Phylogenetic analysis of these samples showed that all positive samples from bat guano were closely related to uncharacterized alphacoronaviruses previously detected in bat guano and/urine samples from southeast Asia (Figure 2). Positive surface samples contained either similar alphacoronaviruses or IBV.

Figure 2. Maximum likelihood phylogenetic tree of coronavirus samples obtained from Bat guano, bat urine and surface swab samples (black) collected from guano farms in Kampong Cham Province, Cambodia in 2024 based on a ~294bp region of the RdRp gene. Reference sequences are shown in green (bat-origin alphacoronaviruses) and red (human betacoronaviruses). Tree was fitted with Neighbor-joining and Tamura-Nei genetic distance model. The outgroup was set as SARS-CoV2 human USA. The nearest 100 matches were recovered from Genbank (NCBI) and pairwise identity used for comparison.

4. Discussion

To our knowledge, our research is unique in examining spillover risks of artificial bat roots to produce bat guano as fertilizer. We employed four methods in our study: surveys on guano production and farming; estimation of bat population size and species determination using carcasses, visual identification, and audio recordings; surveying guano-producing and neighbor households on WASH practices; and testing guano and household food, water, and surfaces using PCR. Bat roost

 doi:10.20944/preprints202603.0997.v1

construction, size, and production were also documented. Findings revealed that bat guano farming is a lucrative enterprise, and farmers are highly knowledgeable. Additionally, while many positive WASH practices were common in households, areas for improvement exist around water treatment, food storage and coverage, and handwashing. Lastly, risks of spillover exist as evidenced by the detection of coronaviruses in guano and urine (15%), surfaces (2.9%), and food (1.4%); though not from water samples (0%). We also discuss recommendations and conclusions from this research.

Bat guano farming is a lucrative enterprise and serves as the primary source of income for many producers. Guano farmers kept detailed records of production and reported earning about 1.25 USD/kg of fertilizer, resulting in a monthly income between 101-200 USD. These economic indicators are consistent with previous findings [2,3]. Farmers had high knowledge of their farms, including accurate predictions of bat species and the timing and direction of emergence flights. Previous researchers have demonstrated variable levels of understanding and knowledge of bats among people with different relationships to bats. High knowledge of bat behavior and ecology was evident in an ethnobiological study in Madagascar, which documented extensive knowledge of bat species among people who interacted with and collected guano from bats [26]. By contrast, farmers in Belize, whose principal interaction with bats was through conflict, exhibited minimal knowledge of ecosystem services provided by bats [27].

Bat guano farms are often passed down to the subsequent generation, indicating that they are a valued cultural and/or economic asset. This aligns with research from another Cambodian community, where participants reported positive perceptions of bats due to their provision of guano, ability to enhance tourism, control pests, and their feasibility as a food source [27]. Despite these positive associations, there were some areas of concern identified, including: 1) bat roosts are located in close proximity to households and neighbors, exacerbating any potential health risk; 2) there was low risk perception and use of PPE among farmers, which aligns with previous research in Uganda and Vietnam [9]; and, 3) gender disparities were reported in guano preparation tasks, which may cause differential risks to household members.

WASH practices within farming households were generally positive, with most households practicing water coverage and treatment, having designated cooking and dishwashing areas, using soap and detergent, and having latrines. However, most households lacked dedicated handwashing stations and had poor food storage practices, including not covering food indoors or while drying. This aligns with another study in Cambodia, where 95% of households did not have handwashing stations and minimal handwashing occurred at key times [28]. Handwashing before and after cooking has also been found to be positively associated with rural farming households' knowledge of zoonoses in Cambodia [29].

Bats are well known as natural reservoirs of many zoonotic viruses, including SARS-CoV, indicating the potential for spillover risk in bat-human interfaces [15,30–33]. Our study found the highest frequency of coronavirus positive samples in bat guano and urine, followed by surfaces, and food. Previous research in Vietnam showed as many as 76.5% of bat guano samples were positive but detected no positives in urine samples [7]. In Sarawak, East Malaysia, 40% of bat guano samples tested positive for coronaviruses including both alpha- and betacoronaviruses [19]. Of guano samples collected in a recent study in Myanmar, 25.8% were positive for a coronavirus [34]. This variability illustrates the dynamic nature of disease transmission in bats and is also likely affected by non-standardized methods for sampling and processing guano for coronavirus testing. Sample positivity rates can also be heavily impacted by UV degradation depending on environmental conditions. Guano has been established as a valuable non-invasive tool for detecting viruses.

The viruses detected in this study do not differ markedly from previous work. Alphacoronaviruses have been detected in *S. kuhlii* in Cambodia and Vietnam [35]. As with this previous work, no betacoronaviruses and no viruses with known public health significance were detected. The finding of IBV was likely attributable to the frequent presence of chickens on farms. Although likely unrelated to the presence of bats, IBV is also a coronavirus, and the detection of IBV

 doi:10.20944/preprints202603.0997.v1

RNA in household surfaces demonstrates the potential for transfer of coronaviruses around farms and into households by farmers and their families.

Our results showed a distinct seasonality of virus shedding in bats, with the greatest sample positivity occurring across a perinatal period. A wide range of seasonal patterns of viral shedding has been recorded in bats, but some, including for some coronaviruses, have shown a similar pattern with greatest shedding occurring around pupping [36]. Shedding of coronaviruses by bats in Cambodia and Thailand showed a pattern of maternal-juvenile transmission during the perinatal period followed by a pulse of increased shedding prevalence among juveniles [15,37]. The coronavirus shedding patterns of *Mormopterus francoismoutoui* in Réunion Island were very similar to those seen in our study, with regular seasonal peaks in coronavirus shedding from just before to just after pupping [38]. While the exact patterns of shedding of specific viruses by host species vary, it is common to find seasonal patterns that align with specific life stages of the host.

The spillover risks identified in our study underscore the need for risk reduction programs. Local spillover risk is driven by the frequency, duration, and intensity of contact between human hosts and wildlife reservoirs [39]. This study indicates clear evidence of close, frequent contacts with bats on, and surrounding, bat guano farms. Specifically, our findings highlight that PPE for farmers, and hygiene risks around handwashing and food covering are critical to address to reduce risks in farmers and their neighbors and communities.

This study had several limitations, namely a small sample area, cross-sectional design, and challenges analyzing samples. All samples came from one region in Cambodia, which limits our understanding of zoonotic disease transmission and spillover risks in other areas of Cambodia and in surrounding countries, such as Thailand and Vietnam. The cross-sectional design does not allow for the investigation of longitudinal patterns in disease transmission, particularly as it relates to seasonality. Lastly, there were challenges in analyzing samples, especially in local laboratories that found sourcing materials for testing difficult. We recommend future research on the role of seasonality in spillover risk, actual viral risk, and evaluations of the effectiveness of culturally appropriate interventions (especially PPE, handwashing, and food storage) to reduce the risk of spillover in farmers and their communities.

Overall, while guano farming offers economic benefits, spillover risks exist. Safely collecting and storing guano, handwashing, and covering foods in guano-producing communities are necessary to mitigate spillover risks. Further research should examine seasonality, attempt to better characterize zoonotic viral risks, and assess intervention effectiveness to ameliorate our understanding and prevent spillover in Cambodia and beyond.

Author Contributions: Conceptualization-Methodology, T. B., B. M. G. and D. L.; Research Data Collection, T. T., S. N., T. R., D. K., S. E. H., and S. O.; Writing—original draft preparation, T. T., S. N.. and T. H.; writing—review and editing, S. T., V. D., B. M. G., C. C., D. N., T. B., and D. L.; supervision, J. G., T.B., and D. L.; project administration, F. N., and H. A.; funding acquisition, H. A., F. N. and J. G. All authors have read and agreed to the published version of the manuscript.".

Funding: This research was funded by the United States Agency for International Development (USAID), grant number 7200AA20RFA00007, led by Tufts University.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. The two study protocols were approved by the Tufts Health Sciences Institutional Review Board (IRB) and the National Ethics Committee for Health Research (NECHR) of Cambodia, the bat ecology and prevalence of pathogens carried by bats protocol (STUDY00003360, No. 043, February 10, 2023), and food, water, and household surfaces assessment of viral presence and spillover risk (STUDY00003488, No. 133, April 11, 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Acknowledgments: This research was made possible by the generous support of the American people through the United States Agency for International Development (USAID). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government. Collaboration of Royal Government of Cambodia's partner agencies significantly contributed to the realization of this research. However, the contents, views and mistakes belong to the authors. The project team thanks the communities for their generosity and willingness to work with the Strategies to Prevent (STOP) Spillover team and other partner organizations. To the guano farmers and their neighbors, we are deeply indebted, and we thank them for their participation in this work.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

IBV	Infectious Bronchitis Virus
NCBI	National Center for Biotechnology Information
PPE	Personal protective equipment
PCR	Polymerase chain reaction
WASH	Water, sanitation, and hygiene

Appendix A

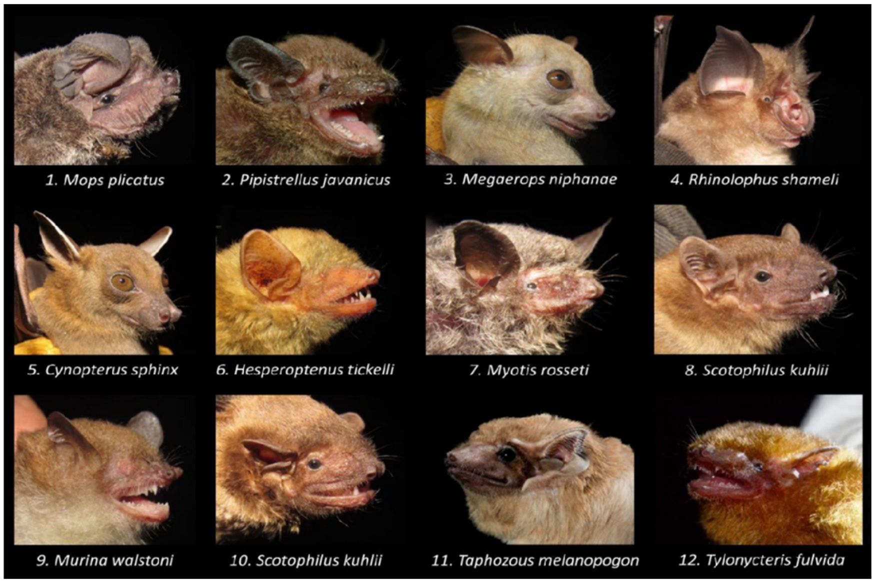

Figure A1. Portraits of Cambodian bat species employed in interviews. Image Credits: Neil Furey (images 1-10,12), Merlin Tuttle (image 11).

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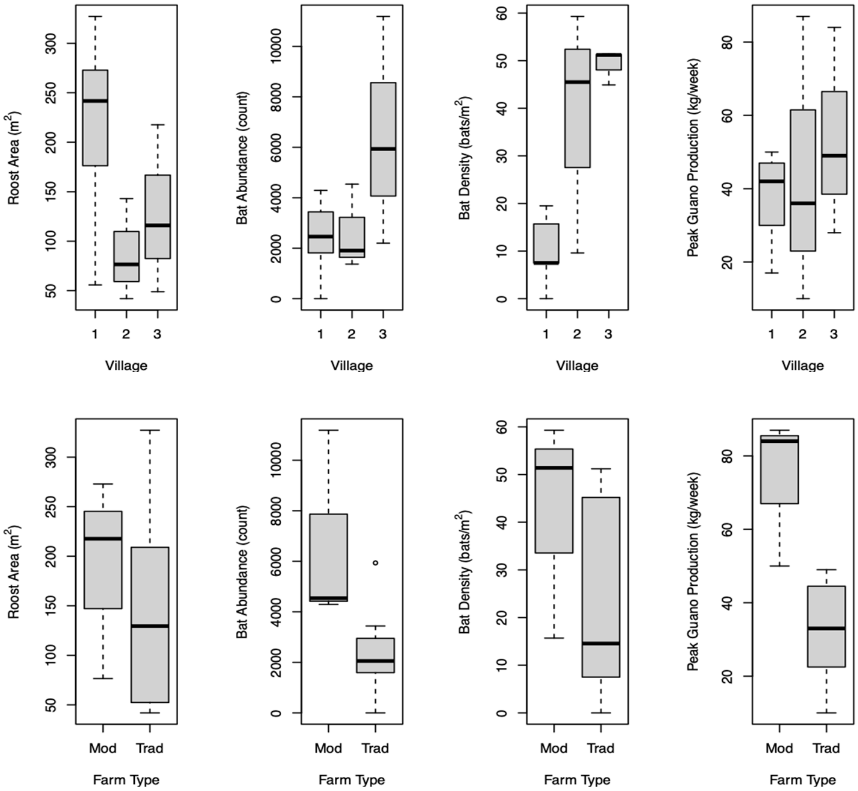

Figure A2. Box and whisker plots showing (from left to right) roost area, bat abundance, bat density and peak weekly guano harvest from guano farms in Kampong Cham, Cambodia in 2023. Top row is separated by the three villages in the study area, the bottom row by traditional (Trad) vs modern (Mod) material roost types.

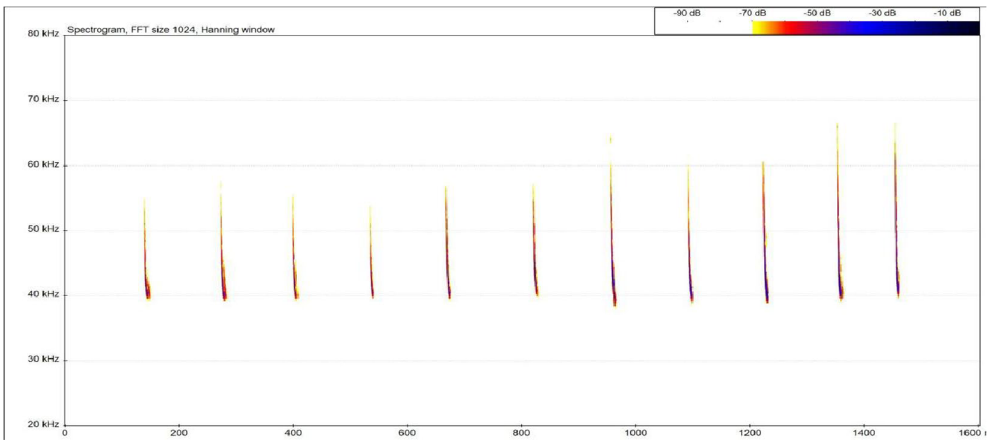

Figure A3. Example spectrogram of search phase calls emitted by S. kuhlii collected at a bat guano farm in Kampong Cham, April 2023.

Table A1. Positivity rate of individual farm by sample type.

ID Farm	Positive samples of feces and urine N (%)		
	Feces	**Urine**	**Total**
1	2 (0.13)	0 (0.00)	2 (0.07)
2	1 (0.08)	0 (0.00)	1 (0.05)
3	0 (0.00)	-	0 (0.00)

4	5 (0.14)	0 (0.00)	5 (0.10)
5	1 (0.13)	-	1 (0.13)
6	3 (0.12)	0 (0.00)	3 (0.09)
7	2 (0.33)	-	2 (0.33)
8	0 (0.00)	-	0 (0.00)
9	2 (0.12)	7 (0.44)	9 (0.27)
10	3 (0.17)	0 (0.00)	3 (0.13)
11	2 (0.09)	1 (0.14)	3 (0.10)
12	2 (0.13)	4 (0.40)	6 (0.24)
13	2 (0.50)	-	2 (0.50)
14	2 (0.25)	1 (0.20)	3 (0.23)
15	7 (0.25)	0 (0.00)	7 (0.21)
16	2 (0.13)	1 (0.11)	3 (0.13)
17	2 (0.11)	5 (0.45)	7 (0.24)
Total	38 (0.146)	19 (0.173)	57 (0.154)

Table A2. Virus strains found in feces and urine in bat guano farm

No.	Virus Strain	Frequency
1	Alphacoronavirus sp. strain VZ_AlphaCoV_16715_47_c2, complete genome	18
2	Alphacoronavirus sp. strain VZ_AlphaCoV_16715_56, complete genome	1
3	Alphacoronavirus sp. strain VZ_AlphaCoV_16715_61, complete genome	3
4	Alphacoronavirus sp. strain VZ_AlphaCoV_16715_63, complete genome	2
5	Alphacoronavirus sp. strain VZ_AlphaCoV_16715_7, complete genome	9
6	Alphacoronavirus sp. strain VZ_AlphaCoV_16715_77, complete genome	2
7	Alphacoronavirus sp. strain VZ_AlphaCoV_16715_86, complete genome	3
8	Alphacoronavirus sp. strain VZ_AlphaCoV_16845_47, complete genome	13
9	Alphacoronavirus sp. strain VZ_AlphaCoV_17819_22, complete genome	1
10	Coronaviridae sp. isolate 75-55-L06-R2-CoV-BAT-VN RdRp gene, partial cds	1
11	Coronavirus PREDICT CoV-35 PREDICT_CoV-35/VN13F0010 F3R RNA-dependent RNA polymerase mRNA, partial cds	2
12	Scotophilus bat coronavirus 512 2005/PREDICT_VN13F0043 RNA-dependent RNA polymerase mRNA, partial cds	1
13	Scotophilus bat coronavirus 512 2005/PREDICT_VN13F0204 F1R RNA-dependent RNA polymerase mRNA, partial cds	1
Total		57

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