



Original Contribution

Seasonality of Date Palm Sap Feeding Behavior by Bats in Bangladesh

Ausraful Islam,¹ Clifton McKee,² Probir Kumar Ghosh,¹ Jaynal Abedin,³ Jonathan H. Epstein,⁴ Peter Daszak,⁴ Stephen P. Luby,⁵ Salah Uddin Khan,⁶ and Emily S. Gurley^{1,2}

¹International Centre for Diarrheal Disease Research, Bangladesh (icddr,b), IPH Building, Shaheed Tajuddin Ahmed Sarani, Room 302968, Mohakhali, Dhaka 1212, Bangladesh

²Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205

³Insight Centre for Data Analytics, National University of Ireland Galway, Galway, Ireland

⁴EcoHealth Alliance, New York, NY

⁵Department of Medicine, Division of Infectious Diseases & Geographic Medicine, Stanford University, 300 Pasteur Dr., L-134, Stanford, CA 94305

⁶BlueDot, Toronto, ON, Canada

Abstract: *Pteropus* bats are the natural reservoir for Nipah virus, and in Bangladesh, it is transmitted to people through consumption of raw or fermented date palm sap. Our objective was to understand seasonal patterns of bat feeding on date palm sap at a location where sap is collected year-round. Seven nights each month over three years, we mounted infrared cameras in four trees to observe bats' feeding behavior at date palm trees harvested for fermented sap production. We described the frequency of bat visits, duration of bat visits, and duration of bat-sap contact by month and by year. We captured 42,873 bat visits during 256 camera-nights of observation, of which 3% were *Pteropus* and 94% were non-*Pteropus* bats. Though the frequency of *Pteropus* bat visits to each tree/night was much lower than non-*Pteropus* bat visits, *Pteropus* bats stayed in contact with sap longer than non-*Pteropus* bats. Frequency of bat visits was higher during winter compared to other seasons, which may arise as a consequence of limited availability of food sources during this period or may be related to seasonal characteristics of the sap. Seasonal alignment of sap consumption by humans and bats may have consequences for viral spillover into humans.

Keywords: Fruit bats, Date palm tree, Infrared camera, Nipah virus, Food contamination, *Pteropus* bats, Non-*Pteropus* bats

INTRODUCTION

Nipah virus spillovers into human populations from pteropodid fruit bats have repeatedly been identified in Asia since 1999. The virus was first described during 1998–1999 as the cause of outbreaks in humans and pigs in Malaysia and Singapore (CDC 1999a; b). A few of the

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Correspondence to: Ausraful Islam, e-mail: islam_ausraf@icddr.org

recognized spillovers involved intermediate hosts like pigs in Malaysia and Singapore and horses in the Philippines (Mohd Nor et al. 2000; Chan et al. 2002; Ching et al. 2015). The majority of reported spillovers have occurred in Bangladesh, where transmission occurs directly from bats to people through consumption of raw or fermented date palm sap (Luby et al. 2009; Islam et al. 2016), which is likely contaminated with virus from bat saliva or urine (Khan et al. 2010). Date palm sap is an agricultural product that has been harvested in Bangladesh for centuries (Ahmed 1995), providing bats with access to this food source that would otherwise be unavailable. Prior studies of bat date palm sap feeding behavior showed that bats can contaminate the sap by licking and in rare cases by urinating directly into sap collection pots (Khan et al. 2010). Nipah virus RNA has been detected in both saliva and urine of *Pteropus* bats (Chua et al. 2002; Wacharapluesadee et al. 2010), and serologic evidence from Bangladesh suggests that *Pteropus medius* frequently had IgG antibodies against Nipah virus, though few bats were shedding virus in saliva or urine at any given time (Epstein et al. 2020).

Each spillover of Nipah virus into humans provides an opportunity for the virus to adapt to a human host, or for a more highly transmissible strain to emerge. Although promising efforts are underway to develop therapeutics (Lo et al. 2019) and vaccines (Geisbert et al. 2021) for Nipah virus, neither is currently available. Interventions to interrupt transmission from bats to people, including through sap consumption, remain the best public health tool we have currently available to prevent human infection with Nipah virus (Khan et al. 2010, 2012; Nahar et al. 2017).

A better understanding of seasonal patterns in bat feeding behavior around date palm sap consumption could be valuable for efforts to prevent spillovers in Bangladesh. While fresh sap consumption by people only occurs during the cooler winter months (Chowdhury et al. 2008), fermented sap, or “*tari*” is consumed year-round and has also been associated with human Nipah infections (Islam et al. 2016). In fact, the only village where spillovers have been recognized across multiple years was known for fermented sap production (Islam et al. 2016). To make *tari*, fresh sap is left in the tree to ferment in pots with growing yeast, so the fermented sap is always being mixed with the fresh (Khan et al. 2012). Despite the potential year-round risk through consumption of fermented sap, surveillance for Nipah spillover events has focused on the winter months when people drink the sap fresh. If bats contaminate fermented sap year-round, these cases could be missed by

surveillance and under-appreciated as part of the spillover mechanism. A better understanding of the seasonal distribution of risk to humans could inform changes to surveillance strategies.

Prior studies of bat feeding behavior related to date palm sap were limited because they only investigated bat feeding during winter months (Khan et al. 2010, 2012). The objective of our study, therefore, was to characterize bats’ feeding behavior at date palm trees harvested year-round for *tari* production, over multiple years, to identify seasonal patterns.

METHODS

We conducted this study at Rangpur District, situated in the northwestern part of Bangladesh, where multiple spillovers of Nipah virus have previously been identified (Islam et al. 2016). For three years, from March 2013 to February 2016, we mounted motion sensor-tripped infrared cameras (Reconyx Hyerfire HC500) to capture bat visits to date palm trees being harvested for sap. The cameras were focused at the shaved surface, sap stream, and the collection pot hanging from the tree, and if there was any movement, took one picture per second for the next five seconds; continued movement extended the photography sequence until the subject exited the frame. The cameras also had an built-in temperature sensor that logged the ambient temperature every time a photograph was taken. The cameras were set from 5:00 PM in the evening until the team returned at 6:00 AM the next morning to download the photographs.

Every month, our target was to collect data on bat visits at four separate trees where sap was being collected for seven consecutive nights. As part of their regular harvesting practice, trees are allowed a resting period from sap collection, at regular intervals. Therefore, the specific trees that we observed changed from month to month. In total, we used 91 date palm trees for our study, and each was marked with a metal sticker with a unique number to link the tree to data about the tree’s height, approximate age, and the area of the shaved surface, as these could be related to the number of bat visits each tree received. To estimate the approximate age of each tree, we assumed that the tree was approximately five years old the first time it was shaved to collect sap (Rana et al. 2009) and then added one year for each additional shaved mark we observed since trees are

shaved once per year. We measured the length and width of the shaved area to calculate the area of this surface.

A trained field research officer at icddr,b (formerly named the International Centre for Diarrhoeal Disease Research, Bangladesh) reviewed the images from the cameras and extracted the data into a spreadsheet, including the total number of bat visits, the number of bats that stayed, the duration of each visit, the duration of bat-sap contact, and ambient temperature. A bat visit was defined as an instance of a bat flying around or landing on the tree, regardless of the duration (Khan et al. 2010). Bat stays were defined as visits where the bat made contact with the tree and stayed for one second or longer, i.e., more than one photograph was taken. Bat-sap contact was defined as a bat touching or licking either the shaved surface of the tree or the date palm sap collection pot (Khan et al. 2010). All images (1560 bat visits) with a duration of stay for five minutes or above were rechecked, and 307 bat visit images needed correction with duration of stay. All bat visits were further categorized by species of bat. There are three known species of fruit bats in Bangladesh, *Pteropus medius* (previously *Pteropus giganteus*) (Mlikovsky 2012), *Rousettus leschenaultii*, and *Cynopterus sphinx* (Ahmed et al. 2009). We could distinguish between *Pteropus* and non-*Pteropus* bat visits due to the larger body size of *Pteropus* bats and their distinct facial appearance; however, we could not differentiate among the non-*Pteropus* species due to similarities in morphology (Ahmed et al. 2009). In some photographs, we were unable to determine the species due to the presence of heavy fog or blurry images; these observations were analyzed separately from the other species groups (Fig. 1) (Khan et al. 2010, 2012).

For the analysis, we divided each year into four seasons: winter (December–February), spring (March–May), monsoon (June–September), and post-monsoon (October–November) (Salam et al. 2003). Our analysis focused on the number of bat stays, i.e., visits with tree contact and a visit duration of one second or more, since the majority of bats were identifiable to species group for that variable (Table S1) and because contact with the tree is more directly relevant to feeding behavior and sap contamination. Similar patterns were observed when all bat visits were analyzed, including those with duration < 1 s. Throughout the text, bat visits generally refer to the more restrictive set of bat stays, unless otherwise indicated. Initial inspection of the data showed that the number of bat visits each night was heavily skewed, with few nights when many bat visits were observed (Figure S1). The duration of bat visits

and bat-sap contact was similarly skewed, with durations ranging from a few seconds to hours. Due to the highly skewed distributions, we estimated mean and 95% confidence intervals (CI) by month and season using a negative binomial distribution for bat visits and an inverse Gaussian distribution for the duration of bat visits and bat-sap contact. These estimates were produced separately for *Pteropus* and non-*Pteropus* bats. Post hoc pairwise tests for differences in the mean number of visits, duration of visits, and duration of bat-sap contact by species group and by season utilized these same distributions and were calculated with the *emmeans* package in R, with Tukey adjustments for multiple comparisons. Since research effort was kept constant across months, variation in season length informed the estimation of means (visits, visit duration, sap contact duration) and associated statistical analyses, but summaries of the total number of bat visits or the cumulative duration of bat-sap contact across seasons did not adjust for season length.

For *Pteropus* and non-*Pteropus* bats separately, we applied multiple regression to explore the association between season, tree height, area of shaved surface, and days since shaving on the number of bat visits per night per tree. The relationship between tree height and tree age showed a strong positive correlation, so we chose to use only tree height as a covariate to avoid issues of collinearity (Figure S2). Additionally, due to a strong relationship between temperature and months and seasons, we did not include temperature as a covariate in our models in combination with season to avoid issues of multicollinearity of covariates (Figure S3). However, we did assess the additive effect of minimum nightly temperature on bat visits during the winter season.

A generalized linear model with all candidate covariates was fit assuming a negative binomial distribution for the number of bat visits. Model selection then proceeded in two stages. First, the model with all covariates and all component subsets were ranked according to the Akaike information criteria adjusted for small sample sizes (AICc). Based on AICc rank, the top models were then subjected to leave-one-out and *k*-fold cross-validation (Molinari et al. 2005). In leave-one-out cross-validation, the model was fit to the data minus one data point and the model was used to predict the remaining point. This process was then repeated for all data points to produce prediction errors for each point, which were summarized as the root mean square error (RMSE) of the predictions. In *k*-fold cross-validation, the data were randomly partitioned into *k*

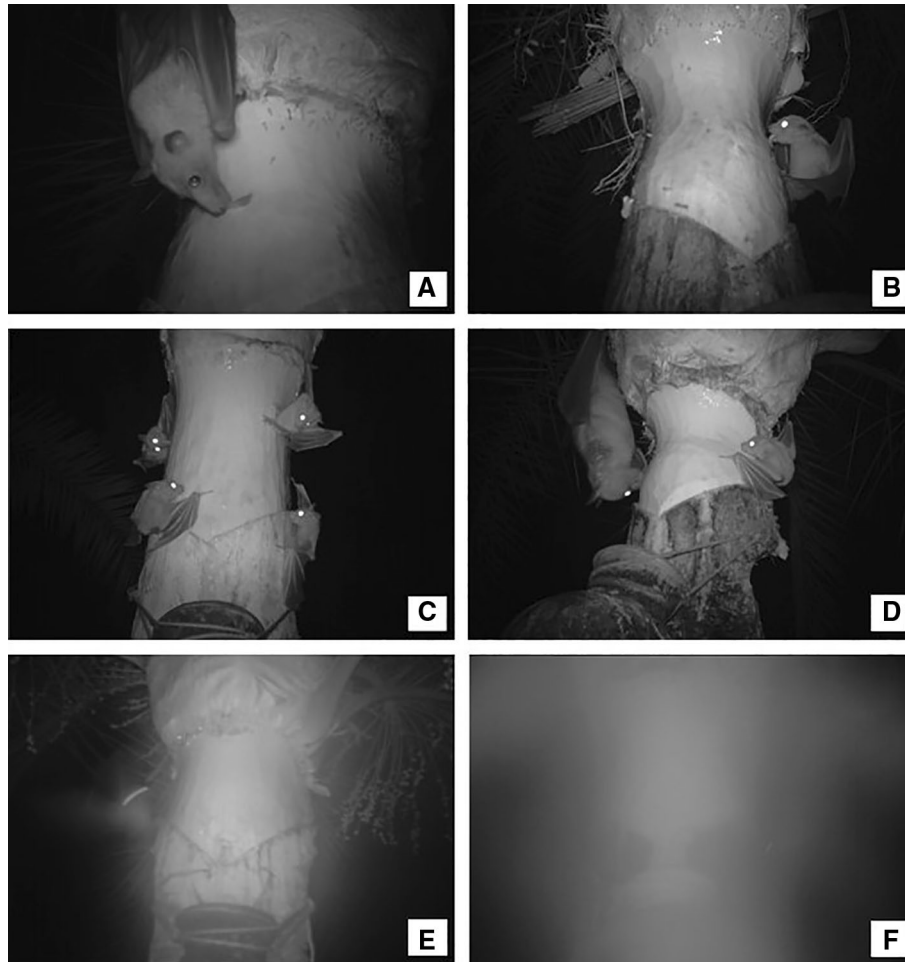


Figure 1. Feeding pattern of a *Pteropus* bat **A**, a non-*Pteropus* bat **B**, group feeding of non-*Pteropus* bats **C**, a mixed group feeding pattern of a *Pteropus* bat and a non-*Pteropus* bat **D**, and unidentified bat images due to blurry picture **E** and presence of fog **F**. Pictures were captured using infrared cameras during our study in Rangpur, Bangladesh, between 2013 and 2016.

groups of equal size. The model was fit on $k-1$ folds and used to predict values of one testing group. This process was then repeated k times, using each k fold once as the testing group. For k -fold cross-validation, we divided the data into ten folds and calculated the RMSE of the predictions. The models for *Pteropus* and non-*Pteropus* bat visits with low AICc and the lowest RMSE from cross-validation were selected as the best models for prediction. To assess the additional effect of minimum nightly temperature on bat visits in winter, we filtered the data to just those observation nights from winter (December–February) and performed model selection as above, replacing season as a candidate covariate with minimum temperature. Thus, the effect of temperature during the winter was estimated while accounting for the other covariates in the selected model. Post hoc tests for the effect of season on

Pteropus and non-*Pteropus* bat visits were performed as above with the R *emmeans* package with Tukey adjustments. Data, R code, and outputs are available at https://github.com/clifmckee/DPS_infrared_camera.

RESULTS

Of the 256 camera-nights of observations over the 36-month study period, 72% (184/256) of nights had bats staying at date palm trees for at least one second, 48% (123/256) of nights had *Pteropus* bats staying, 66% (170/256) of nights had non-*Pteropus* bats staying, and 4% (9/256) of nights had unidentified bats staying (Table S1). A total of 42,873 bat visits to date palm trees were captured, among which 33,018 visits lasted ≥ 1 s. A total of 91% (1093/

1195) of *Pteropus* bats, 79% (31,909/40,329) of non-*Pteropus* bats, and 1% (16/1349) of unidentified bats stayed ≥ 1 s. A total of 32,648 instances of bat contact with date palm sap were recorded, of which 3% (962) were *Pteropus* bats and 97% (31,683) were non-*Pteropus* bats. In 99% (951) of *Pteropus* bats visits, they landed on the branches first and then climbed down to the shaved part while almost 100% (31,624) of non-*Pteropus* bats landed directly on the shaved part. Sixty-seven percent of all bats stayed ≥ 1 s on date palm trees during the winter and 13% during spring. Forty percent of the *Pteropus* bat visits lasting ≥ 1 s occurred during winter and 32% during spring. By contrast, 74% of non-*Pteropus* bats visited stayed ≥ 1 s on date palm trees during winter and 13% during spring (Table S2). Across all nights of the study, visits by both *Pteropus* and non-*Pteropus* bats peaked between the hours 0:00–4:00 (Fig. 2).

Over the 36 months of the study, there was a mean of 1.1 (CI: 0.9, 1.3) *Pteropus* bat visits and 31 (CI: 27, 36 visits) non-*Pteropus* bat visits per tree observation night (Table 1). *Pteropus* bats stayed longer at the trees on average compared to non-*Pteropus* bats: 15.6 min (CI: 3.9, 27.3) versus

31 s (CI: 30, 32). Similarly, *Pteropus* bats stayed in contact with sap longer than non-*Pteropus* bats: 2.5 min (CI: 1.7, 3.3) compared to 31 s (CI: 30, 32 s). *Pteropus* bat visits varied widely across months, with a greater number of total visits across all nights in January and April (> 200) than other months (< 150) (Figure S4). *Pteropus* bat visits were more frequent in winter compared to spring, monsoon, and post-monsoon, although the difference between winter and spring was not statistically significant ($P > 0.05$) (Table 1; Fig. 3). Non-*Pteropus* bat visits also varied by month, with greater visits in February compared to other months (Figure S4). Non-*Pteropus* bat visits were significantly more frequent during winter compared to all other seasons (Table 1; Fig. 3). There was less variation in the duration of visits and bat-sap contact across months and seasons for both groups of bats (Table 1; Figures S5–S8), so the cumulative duration of bat-sap contact was still highest in winter due to the higher number of visits for both groups of bats during winter (Fig. 4). In all cases, *Pteropus* bats were solitary foragers, while out of the 1017 tree observation nights, 180 nights (18%) had at least one example of non-*Pteropus* bats feeding on sap at the same

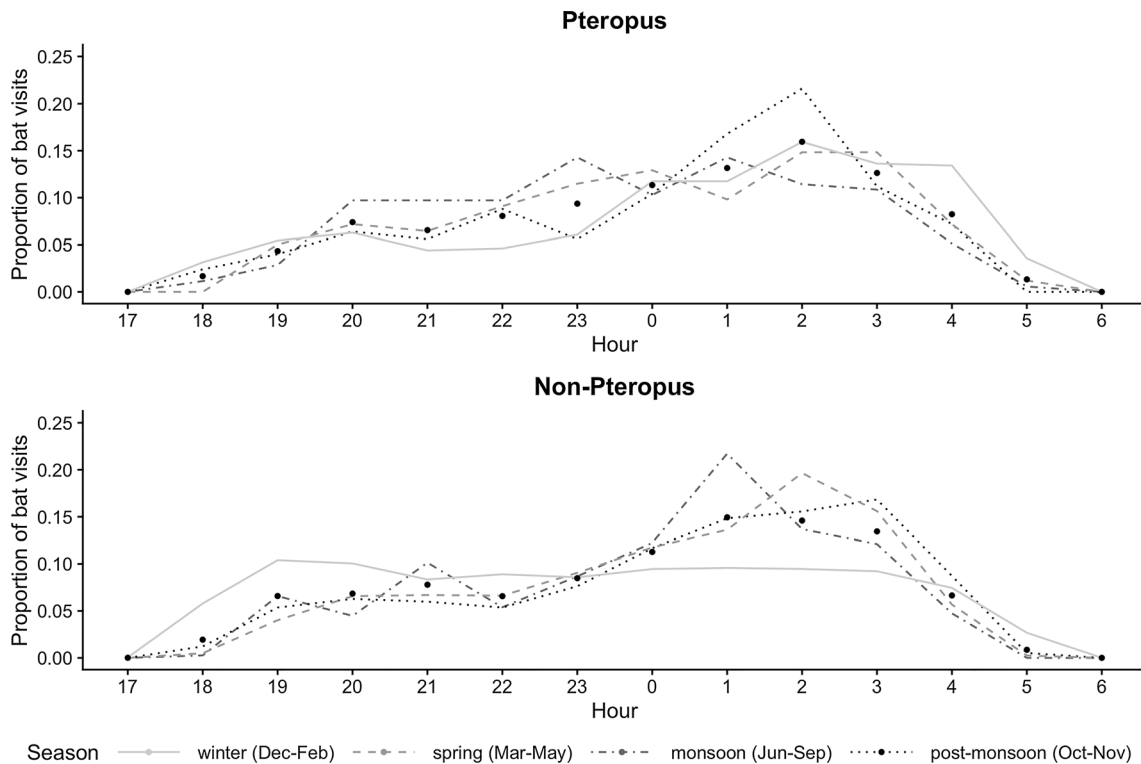


Figure 2. Timing of bat visits during the night aggregated over 256 camera-nights during the three years of the study (2013–2016) in Rangpur, Bangladesh. Bat visits were captured using infrared cameras. The single points show the arithmetic mean proportion of bat visits per hour over all seasons.

Table 1. Comparison of the Frequency of Bat Visits and Duration of Bat-Sap Contact/Visit by Season Collected Using Infrared Cameras over 256 Camera-Nights In Rangpur, Bangladesh, Between 2013 and 2016. Mean Values, 95% Confidence Intervals (CI), and Statistical Significance of Comparisons Between Different Parameters are Derived from Generalized Linear Models, with Bats Staying Per Night Modeled as a Negative Binomial Variable and the Duration of Bat-Sap Contact Modeled as an Inverse Gaussian Variable. Comparison of *Pteropus* vs. Non-*Pteropus* Bats Across all Seasons is Shown in the Last Column.

Seasons	Winter (mean, CI)	Spring (mean, CI)	Monsoon (mean, CI)	Post-monsoon (mean, CI)	Species comparison for all seasons (mean, CI)
<i>Bats staying (bats/night)</i>					
<i>Pteropus</i>	1.7 (1.3, 2.4)	1.4 (1, 1.9)	0.51 (0.35, 0.75)	0.73 (0.46, 1.18)	1.1 (0.9, 1.3)
<i>P</i> value	Reference	<i>P</i> = 0.32	<i>P</i> = 1.1×10^{-6}	<i>P</i> = 0.0029	Reference
Non- <i>Pteropus</i>	94 (61, 144)	16 (10, 25)	1.8 (1.1, 2.9)	21 (12, 35)	31 (27, 36)
<i>P</i> value	Reference	<i>P</i> = 2.4×10^{-8}	<i>P</i> = 1.6×10^{-32}	<i>P</i> = 1.6×10^{-5}	<i>P</i> = 6.9×10^{-158}
<i>Duration of bat-sap contact/visit (seconds)</i>					
<i>Pteropus</i>	129 (107, 152)	147 (116, 178)	184 (121, 247)	200 (113, 287)	151 (102, 199)
<i>P</i> value	Reference	<i>P</i> = 0.36	<i>P</i> = 0.11	<i>P</i> = 0.12	Reference
Non- <i>Pteropus</i>	29 (28, 30)	44 (41, 48)	27 (22, 32)	31 (28, 33)	31 (30, 32)
<i>P</i> value	Reference	<i>P</i> = 5.0×10^{-14}	<i>P</i> = 0.43	<i>P</i> = 0.25	<i>P</i> = 1.3×10^{-6}

time as *Pteropus* and 424 nights (42%) had examples of non-*Pteropus* bats feeding together. The field team recorded the minimum nightly temperature (mean 21°C, range 8–40°C), tree height (mean 614 cm, range 267–1250 cm), shaved area (mean 1124 square centimeters, range 495–1715 square centimeters), and age (mean 20 years, range 11–35 years) of the trees. Bat visits to shaved trees were recorded from between zero to six days after shaving. Trees that had been shaved previously were left to rest, so were not enrolled again in the study.

The best model for *Pteropus* visits included season, tree height, and shaved area as covariates, showing a higher frequency of visits in winter, to taller trees, and to trees with greater shaved area (Fig. 5; Table S3). For example, trees in winter received 3.7 *Pteropus* visits on average if they were 704 cm tall but only 1.4 visits if they were 457 cm tall. The effect of shaved area was smaller (and not statistically significant, $P > 0.05$), with average visits increasing from 2.1 to 2.7 for shaved areas of 960 and 1320 cm², respectively. In this model with additional variables, the difference between the mean number of *Pteropus* visits in winter was significantly greater than in spring and the other seasons. Inspection of the covariates across seasons showed that the trees studied in winter months were shorter on average than trees studied in the other seasons (Figure S9), result-

ing in an upward adjustment of the estimated mean *Pteropus* visits in winter when tree height is included. After accounting for tree height in the best model of winter *Pteropus* visits, bat visits were more frequent on colder nights, with an average of 2.1 visits on nights with a minimum temperature of 8°C and 0.6 visits on nights with a minimum of 30°C (Figure S10). However, this effect was not statistically significant (Table S4).

The best model for non-*Pteropus* visits included season, days since shaving, and shaved area, with more visits in winter, to trees with greater shaved area, and to recently shaved trees, although the effects of shaved area and days since shaving were both not statistically significant (Fig. 5; Table S3). Trees with 960 cm² of shaved area received 67.4 visits in winter versus 87.6 visits to trees with 1320 cm² of shaved area. Trees received an average of 123.8 non-*Pteropus* visits in winter on the day they were first shaved, but this decreased to 47 visits after six days from initial shaving. After accounting for days since shaving in the best model of winter non-*Pteropus* visits, there were significantly more non-*Pteropus* visits on colder winter nights ($P = 0.025$; Table S4). Trees received an average of 87.4 non-*Pteropus* visits on nights with a minimum temperature of 8°C and 16.7 visits on nights with a minimum of 30°C (Figure S10).

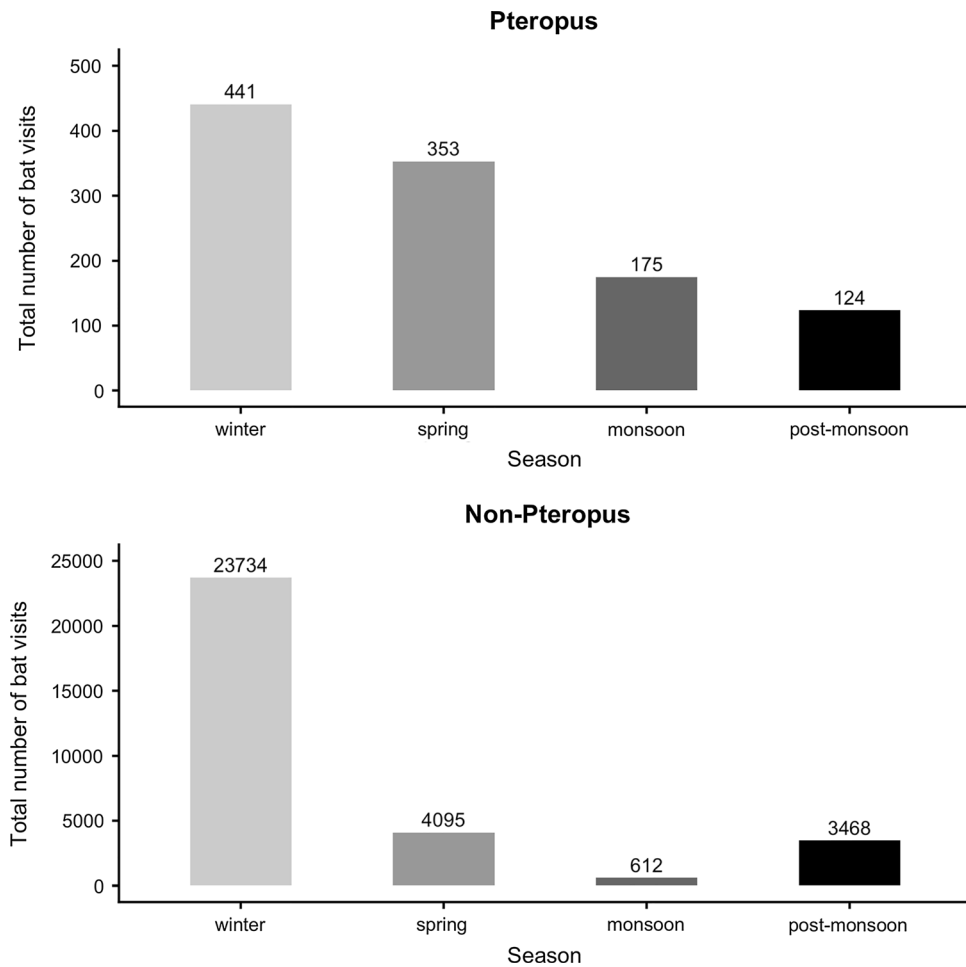


Figure 3. Total observed bat visits by season and species group. Bat visits were captured using infrared cameras over 256 camera-nights during the three years of the study (2013–2016) in Rangpur, Bangladesh. Values show the total visits per season aggregated over all years, not adjusted for the varying length of seasons (winter and spring are three months, monsoon is four months, post-monsoon is two months).

DISCUSSION

All detected spillovers of Nipah virus in humans in Bangladesh resulting from consumption of date palm sap have been detected during December–April (Luby et al. 2006; Luby 2013). Findings from our study show that even when date palm sap is available to bats year-round, *Pteropus* bats still visited these trees more frequently during the months when spillovers are most likely to occur, suggesting that the risk of spillover to humans from fermented sap consumption is concentrated during winter and spring, similar to when fresh sap is consumed. Nevertheless, most reported human Nipah spillover events occur through consumption of fresh sap, which is harvested primarily between mid-October and early April (Annett et al. 1913; Khan et al. 2010). The major ecological driver of spillovers therefore appears to be the convergence of bat and human con-

sumption of fresh sap in winter months. The seasonal patterns of bat feeding on date palm sap, even when the sap is available year-round, deserve further investigation and many biological or ecological factors that modify bat feeding behavior could contribute.

For example, bats may visit the trees more often during the cooler winter months because the sap flows more freely during those months. Previous investigations have shown that date palm sap yields from individual trees peak during the coldest month of January (Annett et al. 1913). There is also some evidence that sap flow is higher on cooler nights during the winter (Annett et al. 1913), patterns that are corroborated by anecdotal reports from sap collectors (Nahar et al. 2010; Islam et al. 2016). The mechanisms that cause sap flow at night and seasonal variation in sap flow are unknown for date palm (*Phoenix sylvestris*) and poorly understood for plants in general (Schenk et al. 2021).

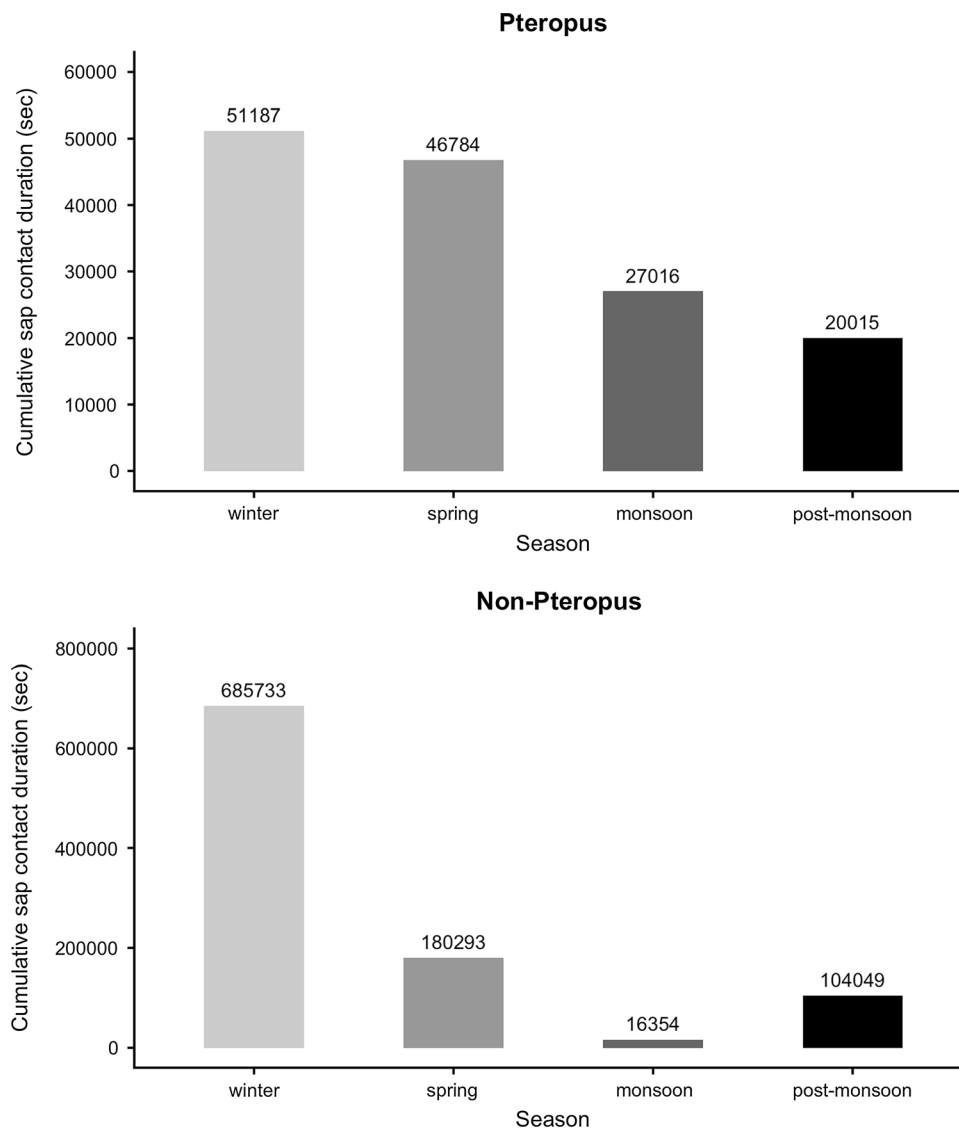


Figure 4. Cumulative duration of bat-sap contact by season and species group. Bat visits were captured using infrared cameras over 256 camera-nights during the three years of the study (2013–2016) in Rangpur, Bangladesh. Values show the sum duration of bat-sap contact over all bat visits in each season aggregated over all years. Values show the total visits per season aggregated over all years, not adjusted for the varying length of seasons (winter and spring are three months, monsoon is four months, post-monsoon is two months).

Positive xylem pressures associated with sap flow in plant tissues have been observed in many plant species, including palms (Davis 1961), but the exact combination of metabolic, physical, and environmental factors that explain positive xylem pressure is not fully characterized, even for well-studied species such as sugar maple (*Acer saccharum*), and appear to be largely dependent on plant anatomy and phenology (Schenk et al. 2021). A plausible hypothesis for positive xylem pressure in palms comes from bamboo, both being pseudo-woody monocots (Schenk et al. 2021). Bamboo species experience diurnal cycles of xylem pressure, with negative pressure during the day and positive

pressure overnight (Wang et al. 2011). Positive pressure in roots could serve as a mechanism for bamboos and palms to refill embolisms that form in xylem tissue during high daytime evapotranspiration, with pressures potentially high enough to refill the stem to the top of the plant (Davis 1961; Yang et al. 2012). Embolism formation, and the subsequent need for refilling from the roots, may be exacerbated during winter months in Bangladesh when precipitation is very low (Cortes et al. 2018). Therefore, high evaporative demand during sunny, low-humidity days in winter followed by clear, cool nights could result in higher xylem pressure that fills up the palm tree, resulting

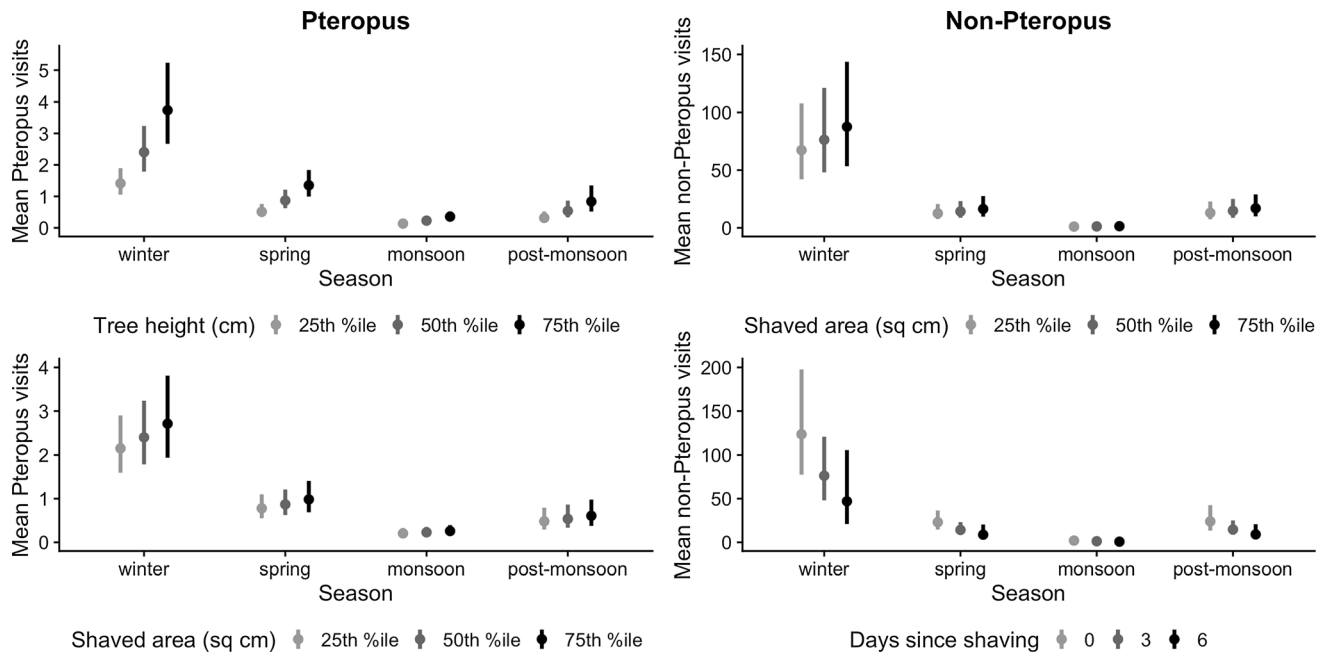


Figure 5. Model estimated mean visits for *Pteropus* and non-*Pteropus* bats during the three years of the study (2013–2016) in Rangpur, Bangladesh. The selected generalized linear model for *Pteropus* visits assumed a negative binomial distribution of visits with season, tree height, and shaved surface on the tree as covariates. Estimated means and 95% confidence intervals of the mean are shown for each season and across three percentiles of the distribution of tree height (457, 592, and 704 cm) and across three percentiles of the distribution of shaved area (960, 1131, 1320 cm²). For estimates in the top *Pteropus* panel, shaved area was fixed at the 50th percentile (1131 cm²); tree height was fixed at the 50th percentile (592 cm) for the bottom panel. The model for non-*Pteropus* visits included season, area of shaved surface on the tree, and days since shaving as covariates. Estimated means and 95% confidence intervals are shown for each season and across three percentiles of the distribution of shaved area (960, 1131, 1320 cm²) and across the minimum, median, and maximum days since shaving. For estimates in the top non-*Pteropus* panel, days since shaving was fixed at three; shaved area was fixed at the 50th percentile (1131 cm²) for the bottom panel.

in greater sap volume. The inverse relationship between yield and sugar content in collected sap might be seen as a direct consequence of daily variation in xylem pressure related to weather conditions and evapotranspiration (Annett et al. 1913). However, additional measurements of sap flow and date palm physiology across seasons are needed to understand the seasonality of sap consumption behavior by bats and humans. Interestingly, more human spillovers of Nipah virus occur following cooler winters (Cortes et al. 2018; McKee et al. 2021), suggesting that weather patterns affecting date palm physiology and sap yield could help to explain annual variation in sap consumption by humans and bats, and thereby the risk of Nipah virus spillover.

It is also possible that bats visit date palm trees more often during these months because the number of other available foods is more limited during winter. Among 49 fruits available in Bangladesh, 35 are available during monsoon, 26 during post-monsoon, 24 during summer, and only 12 during winter, when more than two-thirds of

all bat visits to date palm trees were recorded (Rahman and Rahman 2014). Fruit bats are generalists in that they exploit different food sources, feeding on nectar, pollen, or fruit in one night (Sudhakaran and Doss 2012) and have been identified as opportunistic feeders in different studies (Sudhakaran and Doss 2012; Prasad et al. 2014). As date palm sap is not available for bats in natural settings, we assume that these bats are drinking date palm sap due to its availability, easy access, and high sugar content (Walton and Trowbridge 1983; Rahman and Rahman 2014). Date palm sap is similar in caloric density and macronutrients to other fruit species in the diet of *P. medius*, with high levels of some vitamins and minerals (Barh and Mazumdar 2008; Salvi and Katewa 2012). Thus, date palm sap may act as a key food resource or supplement during a season with reduced availability of other fruits. In Australia, winter seasons when *Pteropus* bats experienced food shortages were associated with a greater risk of Hendra virus spillover in horses, at least in part due to reduced immunological function in bats caused by nutritional stress (Plowright

et al. 2008; Martin et al. 2018). Whether *Pteropus* bats are experiencing similar food shortages and associated immunological stress in winter in Bangladesh has not yet been determined, but will be important to distinguish from other influences on bat visits to date palm trees, such as seasonal differences in sap availability or quantity.

Despite strong seasonal fluctuations in the number of bat visits, *Pteropus* bats visited date palm trees all year round when the sap was available and consumed sap for similar durations across seasons. The sap collected outside of winter is consumed as *tari*, and is partially fermented. *Tari* has been associated with human infections (Islam et al. 2016), further highlighting the year-round risk for Nipah infections associated with *tari* consumption, although the highest risk is during the winter and spring when consumption of fresh sap is more common. Patterns of shedding of Hendra, Ebola, and Marburg viruses from bats appear to be highly seasonal (Pourrut et al. 2007; Amman et al. 2012; Plowright et al. 2015). In contrast with these other diseases, our group found that Nipah virus shedding from *Pteropus* bats is rarely detected in Bangladesh and has no apparent seasonal pattern (Epstein et al. 2020), suggesting that the seasonality of human Nipah spillover events is driven more by overlapping sap consumption by bats and humans during the peak season when trees are tapped for fresh sap than any predictable seasonal transmission dynamics occurring in bats.

Multiple fruit bat species drink date palm sap in Bangladesh, and we observed the same in this study (Khan et al. 2010, 2012). *Pteropus* bats are the known reservoir of Nipah virus in Bangladesh, but serological evidence of Nipah virus was detected among *Rousettus* and *Cynopterus* from other countries (Chua et al. 2002; Wacharapluesadee et al. 2005; Li et al. 2008; Epstein et al. 2016). It is possible that date palm trees serve as points for cross-species transmission of Nipah virus and other pathogens between bat species. The seasonal distribution of visits from non-*Pteropus* bats mirrored the distribution of *Pteropus* species, suggesting that the drivers of these visits are shared across species. Date palm sap contamination is the primary route of Nipah virus spillover from *Pteropus* bats to humans, and may also contribute to infections among livestock in Bangladesh (Luby et al. 2006, 2009; Chowdhury et al. 2014). This may also be a route of spillover for other bat viruses to humans, including viruses carried by non-*Pteropus* fruit bat species. Evidence from encephalitis surveillance in Bangladesh shows that patients who reported recent consumption of date palm sap, but who did not have Nipah

infection, were more likely to die than other patients with encephalitis who did not report sap consumption, suggesting possible zoonotic infection from other viruses (Das et al. 2019).

Pteropus bats spent a longer duration on average during each visit feeding on sap compared to non-*Pteropus* bats, which is consistent with other studies. Studies conducted in Bangladesh and India reported that the average duration of stay for *Pteropus* bats was several times longer than that of non-*Pteropus* bats (Singaravelan and Marimuthu 2004; Khan et al. 2010), likely due to the large differences in their body size and resulting caloric requirements (Storz and Kunz 1999; Perveen and Faiz-ur-Rahman 2014; Kumar et al. 2015). However, there may also be differences in feeding style between the two bat groups. Non-*Pteropus* bats appear to make more visits on average than *Pteropus* in a given night (Table 1). If cameras recorded multiple visits by the same non-*Pteropus* bat in a night, then this might indicate that non-*Pteropus* bats meet their caloric requirements via multiple short-duration visits while *Pteropus* bats make fewer visits but consume sap over a longer duration.

In addition to seasonal variation in bat visits, we found that factors related to individual trees were also associated with bat visits, but these factors differed between bat species. *P. medius* foraged at the topmost layer of trees in other studies (Singaravelan and Marimuthu 2004; Sudhakaran and Doss 2012), which is probably due to their wing loading ability (body mass relative to wing surface area) (Elangovan et al. 2004; Hodgkison et al. 2004) and explains the positive association observed between *Pteropus* bat visits and tree height. Prioritizing interventions (e.g., bamboo skirts) (Khan et al. 2010) on taller trees could prevent transmission of pathogens from *Pteropus* bats to date palm sap. Increases in non-*Pteropus* visits were associated with fewer days since shaving and increases with the size of the shaved area. We assume that trees with larger shaved areas attract more visits and that as days pass from the initial shaving, the shaved area produces less sap; the latter has been reported from sap collectors (Chowdhury et al. 2008). All types of bat visits increased as the minimum temperature decreased during the winter season, though the relationship for *Pteropus* bats was not statistically significant. Whether this is due to higher sap production by trees during colder nights, exacerbated nutritional stress in bats, or possibly both, will require further study.

Our findings should be interpreted with caution due to a couple of important limitations. First, the number of bat

visits we counted should not be interpreted as the number of unique bats who visited the tree because of how this was measured. For example, if one bat were to fly repeatedly in and out of the area where the camera's lens was focused, these would be counted as separate visits by our study. We likely greatly overestimated unique visits by smaller bats because of their propensity to fly around an area repeatedly. However, this problem would not hamper our interpretation of seasonal trends or patterns. Nevertheless, additional efforts to mark individual bats so that they can be identified on camera would help to clarify differences in feeding visits or duration between bat species groups. Second, our observations come from one small area in Bangladesh and cannot be interpreted as representative of bat feeding behavior across the country. However, the locality we chose is associated with Nipah virus spillovers, and the trees we sampled were previously harvested for fresh sap and molasses; therefore, our observations are relevant for the dynamics of Nipah infection. Trees used for *tari* production may differ from trees used for raw sap production in ways that are not accounted for in this study, such as tree height or management practices, which could have also influenced the bat feeding behavior we observed.

CONCLUSION

Using preventive measures to protect sap from bat contamination, like using bamboo skirts, should be a priority for public health intervention efforts and could be used whenever date palm sap is collected (Khan et al. 2012; Nahar et al. 2017). These interventions could also prevent transmission of other viruses from other fruit bats to humans through this route (Khan et al. 2012). Healthcare professionals should be aware that Nipah virus could be the cause of encephalitis cases, even outside of the winter season. Better explaining the seasonal drivers of sap consumption by bats could be important for prevention efforts, and will require additional knowledge about the biology of the date palm trees. For example, if increasing bat access to alternative, high-quality food sources during the winter, such as banyan trees (*Ficus benghalensis*, *Ficus religiosa*) (Sudhakaran and Doss 2012; Prasad et al. 2014; Vanlalnghaka 2015; Krishnarathi and Isaac 2016), would reduce bat visits to date palm sap trees, this could be another way to reduce human risk for Nipah virus. Additional investigations to better understand bat feeding behavior across Nipah endemic areas of South Asia would help to

determine if these kinds of ecological solutions could be useful.

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REFERENCES

- Ahmed FU (1995) Traditional agroforestry systems in Bangladesh. *Agroforestry Newsletter* 3:6–9
- Ahmed, Z. U., A. Ahmed, T. A., S. M. H. Kabir, M. Ahmad, Z. N. T. Begum, M. A. Hassan, and M. Khondker, editors. 2009. Encyclopedia of Flora and Fauna of Bangladesh. Asiatic Society of Bangladesh, Asiatic Civil Military Press, 43/10C Swamibagh, Dhaka-1000, Bangladesh.
- Amman, B. R., S. A. Carroll, Z. D. Reed, T. K. Sealy, S. Balinandi, R. Swanepoel, A. Kemp, B. R. Erickson, J. A. Comer, S. Campbell, D. L. Cannon, M. L. Khristova, P. Atimmedi, C. D. Paddock, R. J. Crockett, T. D. Flietstra, K. L. Warfield, R. Unfer, E. Katongole-Mbidde, R. Downing, J. W. Tappero, S. R. Zaki, P. E. Rollin, T. G. Ksiazek, S. T. Nichol, and J. S. Towner. 2012. Seasonal pulses of Marburg virus circulation in juvenile *Rousettus aegyptiacus* bats coincide with periods of increased risk of human infection. *PLoS Pathogens* 8:e1002877.
- Annett, H. E., G. K. Lele, and B. M. Amin. 1913. The date sugar industry in Bengal: an investigation into its chemistry and agriculture. *Memoirs of the Department of Agriculture in India* 2:281–389.
- Barh D, Mazumdar BC (2008) Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date (*Phoenix sylvestris* Roxb.) sap in treatment of anemia. *Research Journal of Medicine and Medical Sciences* 3:173–176
- CDC (1999) Outbreak of Hendra-like virus—Malaysia and Singapore, 1998–1999. *Morbidity Mortality Weekly Report* 48:265–269
- CDC (1999) Update: outbreak of Nipah virus—Malaysia and Singapore, 1999. *Morbidity Mortality Weekly Report* 48:335–337
- Chan KP, Rollin PE, Ksiazek TG, Leo YS, Goh KT, Paton NI, Sng EH, Ling AE (2002) A survey of Nipah virus infection among various risk groups in Singapore. *Epidemiology & Infection* 128:93–98
- Ching PK, Los Reyes MN, Sucaldito E, Tayag AB, Columna-Vingno FF, Malbas GC, Bolo JJ, Sejvar D, Eagles G, Playford E, Dueger Y, Kaku S, Morikawa M, Kuroda GA, Marsh S, Foxwell AR (2015) Outbreak of henipavirus infection, Philippines, 2014. *Emerging Infectious Diseases* 21:328–331

- Chowdhury MSH, Halim MA, Muhammed N, Haque F, Koike M (2008) Traditional utilization of wild date palm (*Phoenix sylvestris* Roxb) in rural Bangladesh: An approach to sustainable biodiversity management. *Journal of Forestry Research* 19:245–251
- Chowdhury, S., S. U. Khan, G. Cramer, J. H. Epstein, C. C. Broder, A. Islam, A. J. Peel, J. Barr, P. Daszak, L. F. Wang, and S. P. Luby. 2014. Serological evidence of henipavirus exposure in cattle, goats and pigs in Bangladesh. *PLoS Neglected Tropical Diseases* 8:e3302.
- Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, Chan YP, Lim ME, Lam SK (2002) Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes and Infection* 4:145–151
- Cortes MC, Cauchemez S, Lefrancq N, Luby SP, Jahangir Hossain M, Sazzad HMS, Rahman M, Daszak P, Salje H, Gurley ES (2018) Characterization of the Spatial and Temporal Distribution of Nipah Virus Spillover Events in Bangladesh, 2007–2013. *Journal of Infectious Diseases* 217:1390–1394
- Das P, Sazzad HMS, Aleem MA, Rahman MZ, Rahman M, Anthony SJ, Lipkin WI, Gurley ES, Luby SP, Openshaw JJ (2019) Hospital-based zoonotic disease surveillance in Bangladesh: design, field data and difficulties. *Philosophical Transactions of the Royal Society B* 374:20190019
- Davis TA (1961) High root-pressures in palms. *Nature* 192:277–278
- Elangovan V, Raguram H, Priya EYS, Marimuthu G (2004) Wing morphology and flight performance in *Rousettus leschenaultii*. *Journal of Mammalogy* 85:806–812
- Epstein JH, Anthony SJ, Islam A, Kilpatrick AM, Ali Khan S, Balkey MD, Ross N, Smith I, Zambrana-Torrel C, Tao Y, Islam A, Quan PL, Olival KJ, Khan MSU, Gurley ES, Hossein MJ, Field HE, Fielder MD, Briese T, Rahman M, Broder CC, Cramer G, Wang LF, Luby SP, Lipkin WI, Daszak P (2020) Nipah virus dynamics in bats and implications for spillover to humans. *Proceedings of the National Academy of Sciences of the United States of America* 117:29190–29201
- Epstein JH, Anthony SJ, Islam A, Kilpatrick AM, Khan SA, Ross N, Smith I, Barr J, Zambrana-Torrel C, Tao Y, Islam A, Quan PL, Olival K, Gurley E, Hossain MJ, Field HE, Fielder M, Briese T, Rahman M, Cramer G, Wang LF, Luby S, Lipkin WI, Daszak P (2016) Nipah virus ecology and infection dynamics in its bat reservoir, *Pteropus medius*, in Bangladesh. *International Journal of Infectious Diseases* 53:20–21
- Geisbert, T. W., K. Bobb, V. Borisevich, J. B. Geisbert, K. N. Agans, R. W. Cross, A. N. Prasad, K. A. Fenton, H. Yu, T. R. Fouts, C. C. Broder, and A. S. Dimitrov. 2021. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. *npj Vaccines* 6:23.
- Hodgkison R, Balding ST, Zubaid A, Kunz TH (2004) Habitat structure, wing morphology, and the vertical stratification of Malaysian fruit bats (Megachiroptera : Pteropodidae). *Journal of Tropical Ecology* 20:667–673
- Islam MS, Sazzad HM, Satter SM, Sultana S, Hossain MJ, Hasan M, Rahman M, Campbell S, Cannon DL, Stroher U, Daszak P, Luby SP, Gurley ES (2016) Nipah Virus Transmission from Bats to Humans Associated with Drinking Traditional Liquor Made from Date Palm Sap, Bangladesh, 2011–2014. *Emerging Infectious Diseases* 22:664–670
- Khan MSU, Hossain J, Gurley ES, Nahar N, Sultana R, Luby SP (2010) Use of Infrared Camera to Understand Bats' Access to Date Palm Sap: Implications for Preventing Nipah Virus Transmission. *EcoHealth* 7:517–525
- Khan, S. U., E. S. Gurley, M. J. Hossain, N. Nahar, M. A. Y. Sharker, and S. P. Luby. 2012. A Randomized Controlled Trial of Interventions to Impede Date Palm Sap Contamination by Bats to Prevent Nipah Virus Transmission in Bangladesh. *PLoS One* 7:e42689.
- Krishnarathi A, Isaac SS (2016) Food and Feeding Ecology of Bat Preferred Plant Species and their Mutualistic Interactions in Tirunelveli District. *Malaya Journal of Biosciences* 3:81–90
- Kumar M, Priya YS, Mathur V, Elangovan V (2015) Roost selection and roosting ecology of fulvous fruit bat, *Rousettus leschenaultii* (Pteropodidae). *International Journal of Advanced Biological Research* 5:62–68
- Li Y, Wang J, Hickey AC, Zhang Y, Li Y, Wu Y, Zhang H, Yuan J, Han Z, McEachern J, Broder CC, Wang LF, Shi Z (2008) Antibodies to Nipah or Nipah-like viruses in bats, China. *Emerging Infectious Diseases* 14:1974–1976
- Lo, M. K., F. Feldmann, J. M. Gary, R. Jordan, R. Bannister, J. Cronin, N. R. Patel, J. D. Klena, S. T. Nichol, T. Cihlar, S. R. Zaki, H. Feldmann, C. F. Spiropoulou, and E. de Wit. 2019. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. *Science Translational Medicine* 11.
- Luby SP (2013) The pandemic potential of Nipah virus. *Antiviral Research* 100:38–43
- Luby SP, Gurley ES, Hossain MJ (2009) Transmission of human infection with Nipah virus. *Clinical Infectious Diseases* 49:1743–1748
- Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, Gurley E, Khan R, Ahmed BN, Rahman S, Nahar N, Kenah E, Comer JA, Ksiazek TG (2006) Foodborne transmission of Nipah virus, Bangladesh. *Emerging Infectious Diseases* 12:1888–1894
- Martin G, Yanez-Arenas C, Plowright RK, Chen C, Roberts B, Skerratt LF (2018) Hendra Virus Spillover is a Bimodal System Driven by Climatic Factors. *EcoHealth* 15:526–542
- McKee CD, Islam A, Luby SP, Salje H, Hudson PJ, Plowright RK, Gurley ES (2021) The ecology of Nipah virus in Bangladesh: a nexus of land-use change and opportunistic feeding behavior in bats. *Viruses* 13:169
- Mlikovsky J (2012) Correct name for the Indian flying fox (Pteropodidae). *Vespertilio* 16:203–204
- Mohd Nor MN, Gan CH, Ong BL (2000) Nipah virus infection of pigs in peninsular Malaysia. *Revue Scientifique Et Technique De l'OIIE* 19:160–165
- Molinari AM, Simon R, Pfeiffer RM (2005) Prediction error estimation: a comparison of resampling methods. *Bioinformatics* 21:3301–3307
- Nahar N, Paul RC, Sultana R, Sumon SA, Banik KC, Abedin J, Asaduzzaman M, Garcia F, Zimicki S, Rahman M, Gurley ES, Luby SP (2017) A Controlled Trial to Reduce the Risk of Human Nipah Virus Exposure in Bangladesh. *EcoHealth* 14:501–517
- Nahar N, Sultana R, Gurley ES, Hossain MJ, Luby SP (2010) Date palm sap collection: exploring opportunities to prevent Nipah transmission. *EcoHealth* 7:196–203
- Perveen F, Faiz-ur-Rahman. (2014) Occurrence of Indian flying fox, *Pteropus giganteus* (Brünnich) (Chiroptera: Pteropodidae) in district Charsadda Khyber Pakhtunkhwa, Pakistan. *International Journal of Fauna and Biological Studies* 1:61–64
- Plowright RK, Eby P, Hudson PJ, Smith IL, Westcott D, Bryden WL, Middleton D, Reid PA, McFarlane RA, Martin G, Tabor GM, Skerratt LF, Anderson DL, Cramer G, Quammen D, Jordan D, Freeman P, Wang L-F, Epstein JH, Marsh GA, Kung NY, McCallum H (2015) Ecological dynamics of emerging bat

- virus spillover. *Proceedings of the Royal Society b: Biological Sciences* 282:20142124
- Plowright RK, Field HE, Smith C, Divljan A, Palmer C, Tabor G, Daszak P, Foley JE (2008) Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). *Proceedings of the Royal Society b: Biological Sciences* 275:861–869
- Pourrut X, Delicat A, Rollin PE, Ksiazek TG, Gonzalez JP, Leroy EM (2007) Spatial and temporal patterns of Zaire ebolavirus antibody prevalence in the possible reservoir bat species. *Journal of Infectious Diseases* 196(Suppl 2):S176–183
- Prasad ER, Dileep P, Aryasree MP, Anjana K, Sunojkumar P (2014) Foraging Behaviour of Indian Flying Fox *P. giganteus* in Kerala. *Annals of Plant Sciences* 3:883–887
- Rahman M, Rahman J (2014) Medicinal Value and Nutrient Status of Indigenous Fruits in Bangladesh. *Nova Journal of Medical and Biological Sciences* 2:1–19
- Rana P, Sohel S, Islam S, Akhter S, Chowdhury MS, Alamgir M, Koike M (2009) Traditional practice of palm husbandry in the southeastern region of rural Bangladesh: status and potentials. *International Journal of Biodiversity Science & Management* 53:155–161
- Salam A, Bauer H, Kassin K, Ullah SM, Puxbaum H (2003) Aerosol chemical characteristics of a mega-city in Southeast Asia (Dhaka, Bangladesh). *Atmospheric Environment* 37:2517–2528
- Salvi J, Katewa SS (2012) Chemical composition and nutritive value of sap of *Phoenix sylvestris* Roxb. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 11:578–583
- Schenk HJ, Jansen S, Hölttä T (2021) Positive pressure in xylem and its role in hydraulic function. *New Phytologist* 230:27–45
- Singaravelan N, Marimuthu G (2004) Nectar feeding and pollen carrying from *Ceiba pentandra* by pteropodid bats. *Journal of Mammalogy* 85:1–7
- Storz JF, Kunz TH (1999) *Cynopterus Sphinx*. *Mammalian Species* 613:1–8
- Sudhakaran MR, Doss PS (2012) Food and foraging preferences of three pteropodid bats in southern India. *Journal of Threatened Taxa* 4:2295–2303
- Vanlalnghaka C (2015) Seasonal variation in the diet of the frugivorous bat, *Rousettus leschenaultia*. *Science Vision* 15:106–114
- Wacharapluesadee S, Boongird K, Wanghongsa S, Ratanasetyuth N, Supavonwong P, Saengsen D, Gongal GN, Hemachudha T (2010) A longitudinal study of the prevalence of Nipah virus in *Pteropus lylei* bats in Thailand: evidence for seasonal preference in disease transmission. *Vector-Borne and Zoonotic Diseases* 10:183–190
- Wacharapluesadee S, Lumlertdacha B, Boongird K, Wanghongsa S, Chanhom L, Rollin P, Stockton P, Rupprecht CE, Ksiazek TG, Hemachudha T (2005) Bat Nipah virus, Thailand. *Emerging Infectious Diseases* 11:1949–1951
- Walton R, Trowbridge BJ (1983) The use of radio-tracking in studying the foraging behaviour of the Indian flying fox (*Pteropus giganteus*). *Journal of Zoology* 201:575–579
- Wang F, Tian X, Ding Y, Wan X, Tyree MT (2011) A survey of root pressure in 53 Asian species of bamboo. *Annals of Forest Science* 68:783–791
- Yang S-J, Zhang Y-J, Sun M, Goldstein G, Cao K-F (2012) Recovery of diurnal depression of leaf hydraulic conductance in a subtropical woody bamboo species: embolism refilling by nocturnal root pressure. *Tree Physiology* 32:414–422