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1 **Nipah virus detection at bat roosts following spillover events in Bangladesh, 2012–2019**

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17 **Abstract**

18 Knowledge of the dynamics and genetic diversity of Nipah virus circulating in bats and at
19 the human-animal interface is limited by current sampling efforts, which produce few detections
20 of viral RNA. We report on a series of investigations at bat roosts identified near human Nipah
21 cases in Bangladesh between 2012 and 2019. Pooled bat urine samples were collected from 23
22 roosts; seven roosts (30%) had at least one sample with Nipah RNA detected from the first visit.
23 In subsequent visits to these seven roosts, RNA was detected in bat urine up to 52 days after the
24 presumed exposure of the human case, although the probability of detection declined rapidly
25 with time. These results suggest that rapidly deployed investigations of Nipah virus shedding
26 from bat roosts near human cases could increase the success of viral sequencing compared to
27 background surveillance and enhance our understanding of Nipah virus ecology and evolution.

28 **Introduction**

29 Nipah virus is an emerging paramyxovirus (genus *Henipavirus*) that has caused outbreaks
30 of neurologic and respiratory disease in humans and livestock in Bangladesh, India, Malaysia,
31 Singapore, and the Philippines (*1–4*). The primary hosts of henipaviruses are fruit bats (family
32 Pteropodidae) in Africa, Asia, and Australia (*5*). Although Nipah virus causes no apparent
33 disease in bats (*6,7*), the case fatality rate in humans can be 40–70% (*2,8,9*). Additionally, Nipah
34 virus has characteristics that enable repeated outbreaks in humans. Its bat hosts are widespread in
35 South and Southeast Asia, regions with dense human and livestock populations (*10*) that could
36 facilitate virus spillover and spread (*11*). Nipah virus can transmit directly from bats through
37 human consumption of date palm sap contaminated with bat saliva, urine, or feces or indirectly
38 via spillover to domesticated animals (*12–14*). Bangladesh has experienced multiple Nipah
39 outbreaks since 2001 with confirmed person-to-person transmission, albeit below the threshold

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40 necessary for sustained epidemics (*8*); however, the virus transmitted rapidly among farmed pig
41 populations in Malaysia, producing infection rates of 100% on some farms, and spread between
42 farms through shipments of infected animals (*15,16*). There are no commercially available
43 vaccines or therapeutics for Nipah virus to prevent or mitigate disease in the case of an epidemic,
44 although this is an area of active research (*17,18*). Finally, Nipah virus is an RNA virus.
45 Although documented genetic diversity within Nipah viruses is limited (*19–23*), the high
46 mutation rates observed in RNA viruses are an important predictor of zoonotic potential (*24*) and
47 could theoretically produce variants with sufficient transmissibility in humans to cause a
48 sustained epidemic (*25,26*). Given the wide geographic range and unsampled diversity of Nipah
49 viruses, it is possible that variants exist and circulate in bats that are more transmissible among
50 humans, and each spillover event is an opportunity for a more transmissible virus to emerge (*27*).
51 Genetic and phenotypic diversity among Nipah viruses exists, but the human health
52 implications of this variation are unclear. Nipah virus genotypes from Bangladesh and India are
53 genetically distinct from Malaysian genotypes (*21–23*). While the Malaysian genotypes are less
54 diverse than those from Bangladesh and India (*23*), genotypes from Malaysia derive solely from
55 pigs, humans, and bats during the 1998-1999 outbreak whereas genotypes from Bangladesh
56 derive from multiple human outbreaks and surveys of bats since 2004. Another widely cited
57 difference is that person-to-person transmission of Nipah virus was observed rarely in Malaysia
58 (*28–30*) whereas in Bangladesh it accounted for a third of reported cases (*8*) and in India, over
59 75% of cases (*1,9,31*). However, person-to-person transmission in Malaysia was not widely
60 investigated beyond healthcare workers and less than 10% of Nipah cases transmit the virus to
61 another person, usually a family caregiver (*8,28*). Some of this variation in transmission mode
62 and severity may be attributable to differences in exposure, sampling, infrastructure, and culture

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63 between countries, but differences between viral strains may explain additional variation. Cases
64 in Malaysia were less likely to present with a cough or difficult breathing and abnormal chest
65 radiographs than cases in Bangladesh (*29,32,33*). These differences in transmissibility and
66 pathogenicity between Nipah virus strains from Malaysia and Bangladesh have been partly
67 confirmed by animal experiments, although with conflicting results (*34–36*). The reviewed
68 evidence suggests that genetic variation in Nipah virus may produce differences in pathogenicity
69 or transmissibility, so it is possible that more transmissible strains of Nipah virus may circulate
70 undetected in bat populations.

71 However, our knowledge of Nipah virus diversity is limited to the few virus sequences
72 obtained to date. According to GenBank and recent studies (*19,23*), only 76 Nipah virus
73 genomes and 153 nucleocapsid protein genes have been sequenced, 51 and 37 of which derive
74 from human patients, respectively. Current research efforts are not optimized to characterize
75 Nipah virus genotypes circulating in bats. Longitudinal surveys indicate that exposure to Nipah
76 virus is high (~40%) in some *Pteropus medius* populations in Bangladesh based on serology, but
77 the prevalence of detectable Nipah virus RNA is low (<5%) at any given time (*37*). In addition,
78 viral loads in collected bat samples are often low (*23*), preventing genetic sequencing or isolation
79 of viruses necessary for describing viral diversity. Sampling methods that increase the success of
80 detecting Nipah virus in bat populations and increase yield so that sequencing is possible would
81 be useful for monitoring genetic changes in this virus. In this study, we focused Nipah virus
82 detection to *Pteropus medius* bat roosts nearby to human cases identified in Bangladesh during
83 outbreak investigations between 2012 and 2019. We aimed to identify whether bat roosts were
84 actively shedding Nipah virus RNA in urine and how long shedding continued after initial

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85 detection. Additionally, we sought to identify characteristics of bat roosts that could be
86 associated with higher likelihood of testing positive.

87 **Materials and Methods**

88 *Nipah virus case investigations*

89 During 2012 to 2019, human cases of suspected Nipah virus infection with a history of
90 date palm sap consumption were identified via surveillance at three hospitals in Faridpur,
91 Rajshahi, and Rangpur Districts in Bangladesh (*38*). Additional suspected cases in other regions
92 were identified year-round from media reports (*39*). Forty-seven primary cases of Nipah virus
93 representing spillover from bats were identified in 2012–2018, of which 17 were investigated in
94 this study. Four additional spillover cases were investigated in 2019, but the total spillovers from
95 that year is unclear due to a lack of reporting. Case exposure to Nipah virus was evaluated with
96 an enzyme-linked immunosorbent assay (ELISA) or PCR (*40*). Investigation teams visited the
97 suspected case villages to gather evidence of case clusters and identify the exposure route (*41*).
98 In some cases, teams were deployed before human cases were confirmed via ELISA or PCR.

99 Teams searched for *Pteropus medius* bat roosts within a 20 km radius of the case house
100 by asking community members about known roost sites and by scouting. Some identified roosts
101 were located on burial grounds or over water bodies and could not be sampled (Appendix Table
102 1). Between 12:00 AM and 4:00 AM, teams placed 4 to 20 polyethylene tarps under each roost,
103 depending on the available area and size of the roost, to collect urine. Tarps were concentrated
104 under branches with denser aggregations of bats. Tarps were approximately 6'x4' in size before
105 2019 and 3'x2' in 2019; this change was made so that fewer bats contributed to urine pools to
106 improve estimates of prevalence (*42*). Between 5:00 AM and 6:00 AM, teams returned to the
107 roosts and collected bat urine from the tarps with a sterile syringe. Urine collected from tarps

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108 was either pooled by individual tarp or mixed together from multiple tarps and then divided into
109 aliquots. We found no significant difference in Nipah detection between the two strategies (see
110 Appendix). Aliquots were tested for Nipah virus RNA at icddr,b or NIH laboratories using
111 quantitative real-time reverse transcription PCR (RT-qPCR) targeting the nucleoprotein gene
112 (*43*). Roosts with Nipah virus RNA detected in any aliquots at the first sampling event were
113 revisited (3–16 days between sampling events) until all aliquots from a roost tested negative.
114 Attempts to culture Nipah virus from RT-qPCR positive samples at NIH yielded no virus
115 isolates; viral culture was not attempted at icddr,b due to the absence of biosafety level 4
116 facilities.

117 *Statistical analysis*

118 For each laboratory-confirmed spillover of Nipah virus in a human, the symptom onset
119 date and the coordinates of the case house were recorded. Teams identified the probable date of
120 patient exposure to Nipah virus via date palm sap consumption for some cases; otherwise, the
121 exposure date was assumed to be seven days prior to symptom onset based on the mean
122 incubation period of Nipah virus for primary cases linked to spillover (*44*).

123 Logistic regression was used to assess features of the roost sites associated with a roost
124 testing positive for Nipah virus at the first sampling visit. Covariates in the model included the
125 number of days between the first case patient exposure to date palm sap and roost sampling, the
126 number of bats in the roost, the distance between the human case house and the roost site, and
127 the number of human spillover cases associated with each nearby roost. Model selection was
128 then performed to choose important features using Akaike's corrected information criterion
129 (AICc) (*45*).

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130 For all roost sites that tested positive for Nipah virus at first sampling, we recorded the
131 number of tested urine aliquots that were positive for Nipah virus at each visit. Since cycle
132 threshold (CT) values from RT-qPCR were not reported for all tests, we used the proportion of
133 positive aliquots as an approximate measure of the intensity of virus shedding in roosting bats,
134 assuming that the roosts with higher concentrations of virus in urine will produce more positive
135 aliquots. We then analyzed changes in the proportion of positive aliquots across roosts along two
136 time axes. First, we aligned dates based on the number of days since presumed exposure date of
137 the first human spillover Nipah case associated with each roost site. We also aligned roost
138 sampling dates to the number of days since the start of the calendar year for comparison.
139 Binomial linear models were fit to estimate the probability of detecting a Nipah virus-positive
140 aliquot at each roost along each time axis.

141 To evaluate the utility of sampling bat roosts nearby to human Nipah cases as a
142 surveillance approach, we compared the rate of successful Nipah virus detections from this study
143 to data recently reported by Epstein et al. (*37*). Samples from that study were collected quarterly
144 from a *P. medius* roost in Faridpur District between 2007 and 2012 as part of a longitudinal
145 study, from visits to different roosts throughout Bangladesh between 2006 and 2011 as part of a
146 cross-sectional spatial analysis, or as part of Nipah outbreak investigations in 2009, 2010, and
147 2012. Urine samples were either collected from individual bats or from underneath roosts. For
148 these comparisons, we considered each roost visit as a discrete sampling event, including repeat
149 visits to the same roost. Ignoring the initial visits to seven roosts nearby five suspected human
150 cases that were Nipah-negative, the 23 roosts in our study were sampled across 47 visits.
151 Comparisons between studies were made for the number of sampling visits with positive Nipah
152 detections and the number of positive urine samples (individual or pooled aliquots from roosts)

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153 across all sampling visits or during the first visit to each roost. Comparisons were evaluated
154 using a chi-squared test of proportions or Fisher's exact test, where appropriate. Statistical tests
155 were considered significant if p-values were <0.05.

156 *Ethical approval*

157 All study participants or proxies provided informed consent before participation and
158 personally identifiable information from case patients was delinked from the data before use.
159 Written permission was obtained from the Bangladesh Forest Department for sampling the bats
160 and team members obtained permission from landowners before sampling roosts. Protocols for
161 case investigations and roost sampling were reviewed and approved by the institutional review
162 board at icddr,b.

163 **Results**

164 Teams investigated roosts near 21 suspected human cases of Nipah virus infection during
165 2012–2019 (Appendix Table 1). The cases were clustered in the central and northwest districts of
166 Bangladesh, close to the three surveillance hospitals (Figure 1). Symptom onset for patients
167 occurred in winter (December–February) with the exception of one case patient in Manikganj
168 District who presented with symptoms in March 2013. No roost investigations were performed in
169 2017 and 2018 due funding constraints.

170 For each case patient, 1–3 *Pteropus medius* roosts were identified within 0–17.9 km of
171 the patient's home (Appendix Dataset 1). Five additional roosts were identified but could not be
172 sampled due to their location on burial grounds or over water (Appendix Table 1). A total of 30
173 roosts were sampled. The first sampling visits occurred 17–62 days after the case patients'
174 exposure to date palm sap, either reported from the case investigation or back-calculated as

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175 seven days prior to the onset of symptoms (Appendix Dataset 1). Five of the suspected patients
176 tested negative for Nipah virus by ELISA or PCR, and the seven roosts identified near the case
177 houses yielded no Nipah virus RNA. Since our interest was in whether sampling around Nipah
178 cases would help to identify roosts with active Nipah virus shedding, the suspected but Nipah
179 test-negative case patients and associated bat roosts were left out of statistical analyses.
180 Sensitivity analyses with these samples included produced statistically similar results. RT-qPCR
181 testing of pooled urine aliquots detected 7/23 (30%) roosts as positive for Nipah virus RNA in
182 one or more aliquots at the first sampling visit.

183 Logistic regression on the presence of Nipah virus RNA in roost urine at the first
184 sampling event was performed on 22 distinct roosts using four explanatory variables; one roost
185 was omitted due to missing data on the number of bats. Although roosts with positive urine
186 aliquots tended to have more associated human Nipah spillover cases, were sampled sooner after
187 patient exposure, were more distant from the case house, and had a smaller number of bats, none
188 of these variables was significantly associated with roost positivity in univariate or multiple
189 regression analyses (Figure 2; Appendix Table 2) and AICc identified the intercept-only model
190 as the best model (Appendix Table 3).

191 For the seven roosts where NiV RNA was detected at least once, data were compiled on
192 the number of urine aliquots that tested positive at each repeated sampling visit. Four of the
193 seven roosts were positive at the first visit only and were subsequently only revisited once. The
194 other three roosts remained positive at 1–2 additional sampling visits, although the proportion of
195 aliquots that tested positive declined rapidly with the time since exposure of the first associated
196 human case (Figure 3). For the two roosts with reported CT values from RT-qPCR, the

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197 proportion of positive aliquots decreased over the repeated sampling visits while CT values
198 increased, suggesting a decline in viral load with time (Appendix Table 4).

199 Fitting a binomial model to the PCR data predicted that the probability of detecting at
200 least one urine aliquot from under-roost sampling as positive for Nipah virus RNA at the time
201 (day 0) the associated case patient was presumably exposed was 0.66 (95% confidence interval
202 (CI): 0.42–0.84) (Figure 3). This probability declined to 0.02 (95% CI: 0.01–0.04) by day 52
203 when the last positive roost aliquots were detected and to 0.01 (95% CI: 0–0.02) by day 65 when
204 the last roost was sampled. We also fit a binomial model using the days elapsed since the start of
205 the calendar year (Appendix Figure 1), but the alignment of the virus detections among the roosts
206 was less clustered on this time axis than the days since patient exposure time axis, and the
207 binomial model did not show a significant trend in virus detection over time.

208 Roost urine samples from the current study and individual urine samples from
209 longitudinally sampled roosts in the Epstein et al. study (*37*) produced similar proportions of
210 positive sampling visits (Table 1, comparison A); the detection rate was also similar if only the
211 first visit to each roost in our study was considered (7/23, 30%). In contrast, the proportion of
212 positive aliquots from all sampling visits was significantly higher in our investigations than in
213 the individual urine samples from longitudinal roosts in Epstein et al. (Table 1, comparison B).
214 The detection rate from our study for positive urine aliquots at the first sampling visit was also
215 higher than the detection rate for individual urine samples collected from eight roosts from a
216 cross-sectional study by Epstein et al. (Table 1, comparison C). The detection rate for positive
217 urine aliquots from our study was substantially higher than the detection rate from similarly
218 pooled urine aliquots from underneath longitudinal and cross-sectional roosts in the Epstein et al.
219 study (Table 1, comparison D). Lastly, outbreak investigations of roosts performed by Epstein et

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220 al. produced a higher detection rate than our own roost investigations (Table 1, comparison E),
221 although only four roosts were visited by Epstein et al. and the same roosts were not repeatedly
222 visited as was done in our study.

223 **Discussion**

224 Nipah virus spillovers from bats occur sporadically in Bangladesh, so surveillance that
225 optimizes viral detection from bat populations is a challenge. In contrast with cross-sectional or
226 longitudinal roost surveillance used previously in Bangladesh (*37*), the roost sampling in this
227 study was triggered by Nipah outbreaks in nearby villages. Our approach identified bat roosts
228 with active Nipah virus shedding at an equivalent rate to background surveillance (*37*), but had a
229 higher detection rate in roost urine on a per sample basis. These results indicate that investigating
230 roosts near spillover cases is more efficient than cross-sectional or longitudinal surveillance for
231 obtaining samples with detectable viral RNA (Table 1). Repeated visits to positive roosts also
232 demonstrated that viral RNA was detectable for weeks after the purported exposure date of
233 human cases, although the proportion of positive urine aliquots declined sharply over time.
234 These data suggest that rapid deployment of teams to identify bat roosts and sample urine could
235 increase the probability of detecting and sequencing Nipah virus. Used in combination with
236 longitudinal sampling of bat roosts and surveillance of human or domesticated animal cases, this
237 method could enhance our understanding of Nipah virus dynamics and genetic diversity in bat
238 populations.

239 Furthermore, this study provides important knowledge about the timing of Nipah virus
240 shedding in bats in Bangladesh. Longitudinal surveys have shown that Nipah virus shedding
241 from bats is sporadic throughout the year (*37*), so the peaks in viral detection in roost urine from
242 our study likely coincided with shedding events. However, because these shedding events were

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243 occurring during winter when date palm sap is harvested for human consumption, bat visits to
244 date palm trees to consume sap may be more likely to contaminate sap with virus and lead to
245 human infections (*46*). This suggests that the intensity of shedding events in bats occurring in
246 winter could help to explain some of the spatial and temporal variation in the number of human
247 spillovers that occur in Bangladesh each year (*41*), although more data on the frequency and
248 timing of shedding events and human sap consumption patterns will be needed to fully
249 understand the dynamics of Nipah virus spillover.

250 Our findings come with several caveats due to limitations in our sample size and the
251 study design. Our analysis of factors associated with a roost testing positive at the first sampling
252 visit was unable to identify any significant relationships likely due to low statistical power. We
253 also did not systematically attempt Nipah virus isolation or sequencing in all positive samples, so
254 we cannot estimate the probability of successful isolation or sequencing. However, Nipah virus
255 isolates and sequences have been obtained from some of the roost urine samples included this
256 study. One of the positive roosts sampled in Joypurhat in 2012 produced nine nucleocapsid
257 sequences (GenBank accession numbers: MT890702-MT890710; Rahman et al. (*23*)) and the
258 positive roost in Manikganj from 2013 produced ten virus isolates with full-genome sequences
259 (GenBank accession numbers: MK575060-MK575069; Anderson et al. (*20*)). In fact, of the 39
260 Nipah virus sequences obtained from bats in Bangladesh, 28 (72%) came from urine collected
261 underneath roosts and 24 (86%) of those urine samples came from roost investigations near
262 human cases (Appendix Dataset 2). These patterns suggest that roost urine, especially from
263 roosts near human spillover cases, may contain sufficient Nipah virus for sequencing or culture.
264 Future investigations could track how viral load in roost urine varies during viral shedding

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265 events, which could improve the success of sequencing and isolation and shed light on the
266 ecological and epidemiological conditions that lead to Nipah shedding from bats (*47*).

267 Our case investigations were also limited to the catchment area of three surveillance
268 hospitals and to the winter seasonality of Nipah spillover surveillance. This design systematically
269 misses virus shedding events at bat roosts outside of the surveillance area or during seasons
270 when people are not drinking fresh date palm sap (*13*). Acknowledging that the logistical
271 constrains of our surveillance approach cannot capture all Nipah virus genotypes circulating in *P.*
272 *medius* across Bangladesh, increasing the number of detections is still important, especially
273 given the few Nipah isolates currently available (n = 11). Reactive roost investigations could be
274 complemented with additional roost surveys outside of surveillance areas to understand Nipah
275 virus transmission and genetic diversity in bat populations across Bangladesh.

276 This study provides proof of concept that reactive investigations of bat roosts nearby
277 human Nipah cases can complement ongoing surveillance efforts and could increase the
278 likelihood of viral detection and sequencing. Improvements in virus detection would aid in
279 characterizing the genetic diversity of Nipah viruses circulating in bats and identify novel
280 genotypes that might pose a pandemic threat. Furthermore, these data provide evidence that viral
281 shedding can continue for weeks after an initial spillover event, posing a hazard for additional
282 contamination. Knowledge of the period over which bats are shedding Nipah virus could be used
283 to deploy public health campaigns more efficiently, such as using barriers to prevent bat access
284 to date palm sap (*48*).

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Table 1. Comparison of Nipah virus detection success from this study with results from Epstein et al. 2020 (*37*).

Test ID	Data from Epstein et al. 2020		Data from this study		Statistical test
A	Positive sampling visits (based on individual urine samples from longitudinal roosts where at least one individual urine sample tested positive)	5/18 (28%)	Positive sampling visits (based on pooled roost urine aliquots where at least one urine aliquot tested positive)*	11/47 (23%)	OR = 0.84 P = 0.76
B	Positive individual urine samples from longitudinal roosts (across 18 sampling visits)	8/1671 (0.48%)	Positive roost urine aliquots from sampled roosts (across 47 sampling visits)*	51/1042 (4.9%)	$\chi^2 = 56.8$ $P = 4.9x10^{-14}$
C	Positive individual urine samples from 8 roosts from a cross-sectional spatial study across districts of Bangladesh	0/555 (0%)	Positive roost urine aliquots from the first visit to 23 sampled roosts*	45/525 (8.6%)	$\chi^2 = 47.5$ $P = 5.4x10^{-12}$
D	Positive roost urine aliquots (from longitudinal roosts and cross-sectional roosts,	2/725 (0.28%)	Positive roost urine aliquots from sampled roosts	51/1042 (4.9%)	$\chi^2 = 29.8$ $P = 4.8x10^{-8}$

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	excluding samples from outbreak investigations)		(across 47 sampling visits)*		
E	Positive roost urine aliquots (from outbreak investigations, n = 4)	19/104 (18.3%)	Positive roost urine aliquots from sampled roosts (across 47 sampling visits)*	51/1042 (4.9%)	$\chi^2 = 27.2$ $P = 1.8x10^{-7}$

OR – odds ratio (from Fisher's exact test).

*Excludes the seven roosts associated with five human cases that initially tested negative for Nipah virus. Statistical tests that included these samples produced similar results.

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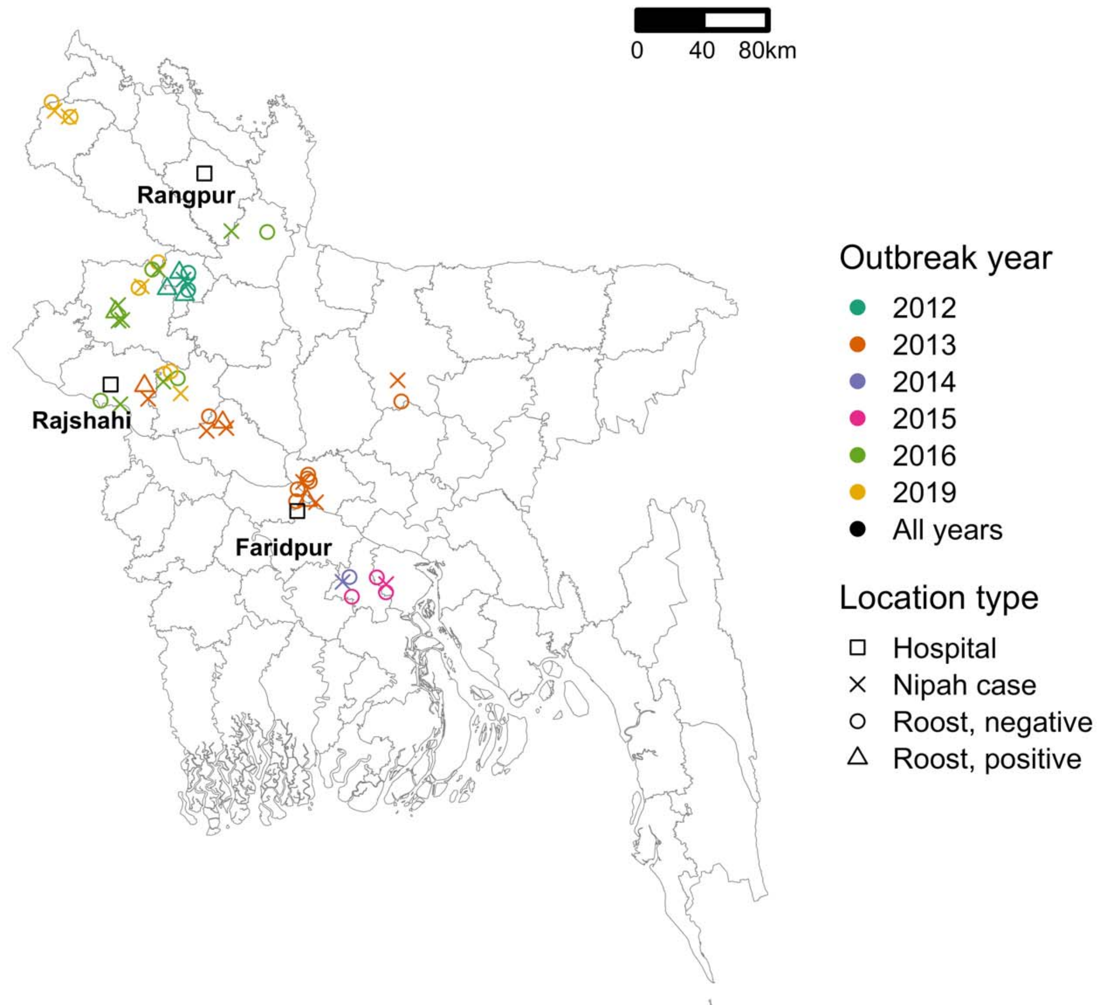

444

445 Figure 1. Locations of human Nipah cases (n = 21) and *Pteropus medius* bat roosts (n = 30)
446 investigated in Bangladesh between 2012 and 2019. Roosts with urine aliquots that tested
447 positive for Nipah virus RNA at the first sampling visit are marked with distinct symbols. Points
448 have been jittered a small amount to increase visibility.

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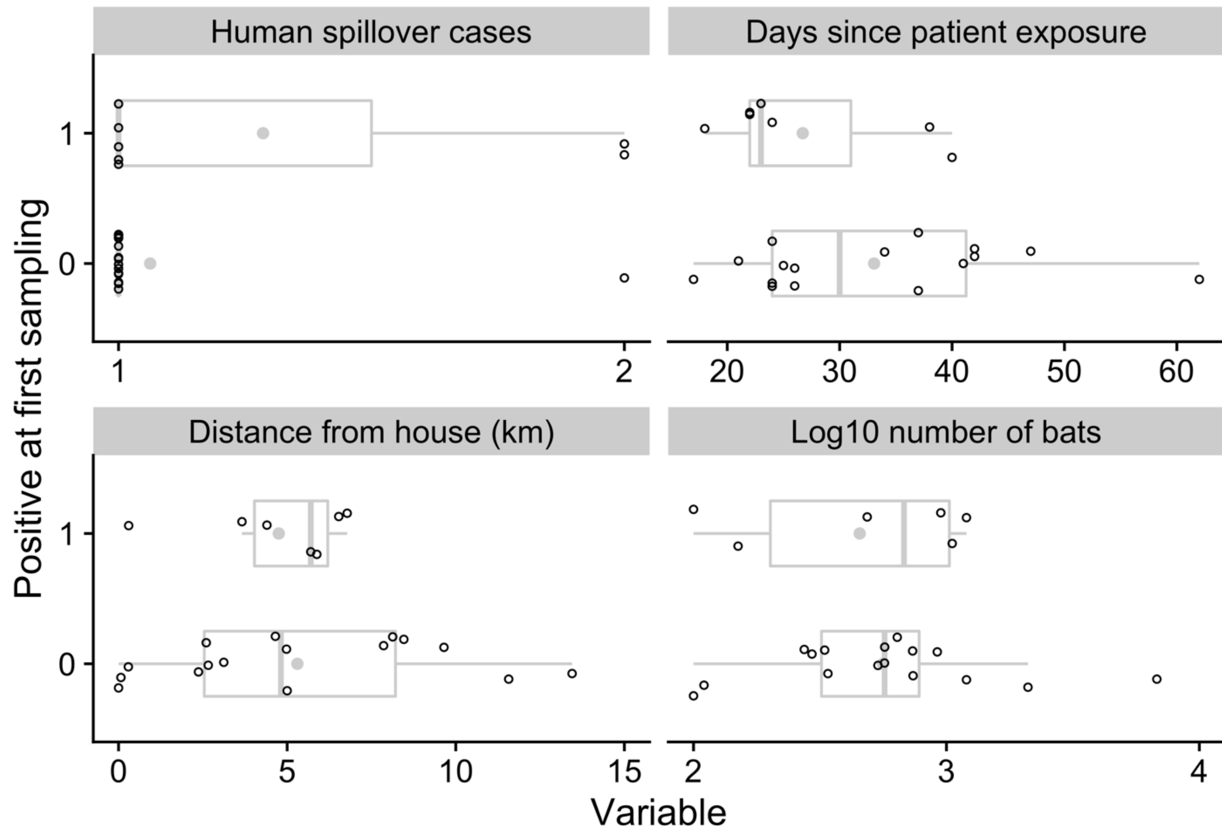

449

450 Figure 2. Descriptive variables for 23 *Pteropus medius* roosts sampled nearby confirmed human
451 Nipah virus cases. Open points show the values associated with the first human case associated
452 with each roost. Means for each variable and positivity status (0 or 1) are displayed as filled gray
453 points.

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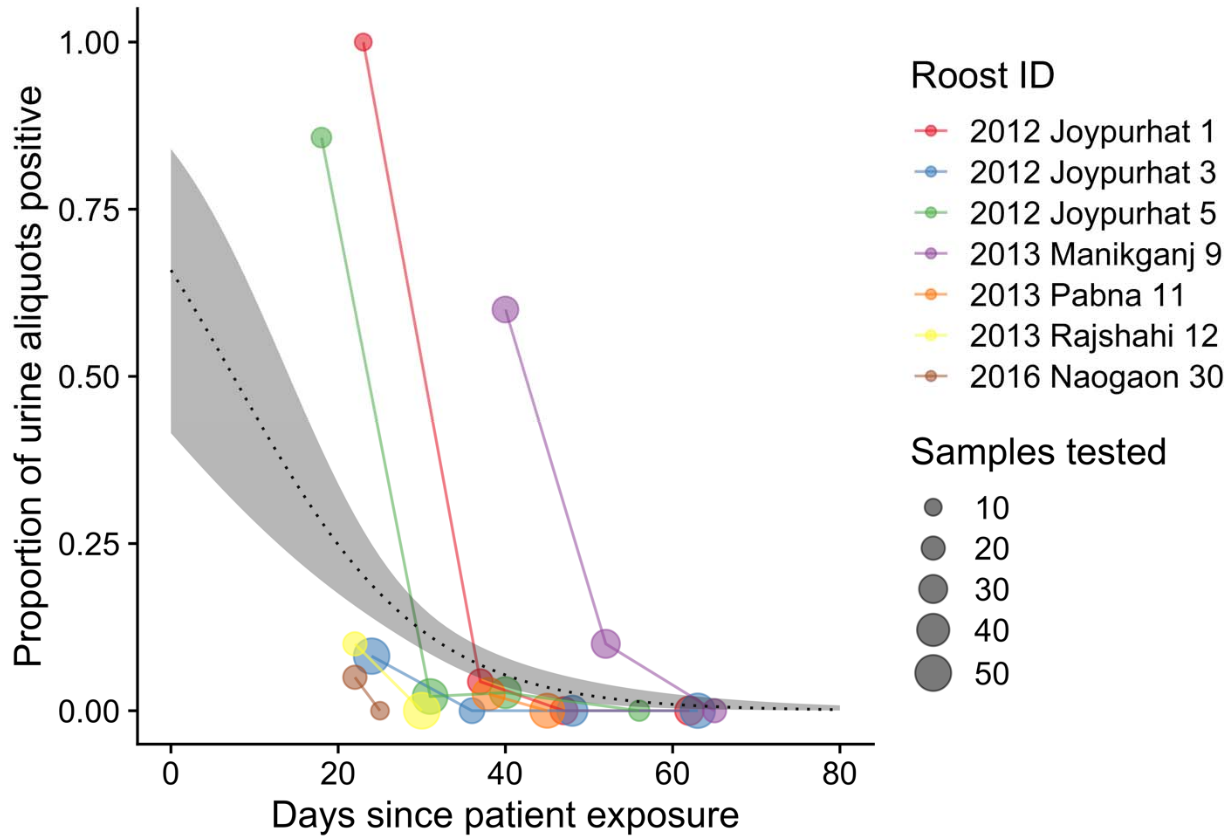

454

455 Figure 3. Results of screening *Pteropus medius* roost urine aliquots for Nipah virus RNA. For
456 each roost, the proportion of urine aliquots out of the total tested (shown by the size of points) is
457 aligned along a time axis of the days since the first associated case patient was exposed to Nipah
458 virus in date palm sap, which was either reported during the investigation or back-calculated as
459 five days prior to reported symptom onset.

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460 **Appendix Table 1.** *Pteropus medius* roosts identified and sampled near suspected human index
461 cases of Nipah virus infection in Bangladesh, 2012–2019. Roosts that were identified but not
462 sampled were not accessible because they were located on burial grounds or over water.

Year	Division	District	Suspected Nipah cases	Total identified roosts	Sampled roosts	Unsampled roosts
2012	Rajshahi	Joypurhat	2	6	5	1
2013	Mymensingh	Mymensingh	1	1	1	0
	Dhaka	Manikganj	1	3	3	0
	Rajshahi	Pabna	2	2	2	0
		Rajshahi	1	1	1	0
2014	Dhaka	Madaripur	1	1	1	0
		Manikganj	1	3	3	0
2015	Dhaka	Madaripur	1	3	3	0
2016	Rajshahi	Joypurhat	1	1	1	0
		Naogaon	3	1	1	0
		Natore	1	1	1	0
		Rajshahi	1	1	1	0
	Rangpur	Gaibandha	1	1	1	0
2019	Rajshahi	Naogaon	1	2	2	0
		Natore	1	5	2	3
		Rajshahi	1	2	1	1
	Rangpur	Thakurgaon	1	1	1	0

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464 **Appendix Table 2.** Multivariate logistic regression model coefficients for the presence of Nipah

465 virus RNA at *Pteropus medius* roosts (n = 22) near human cases.

Variable	Description	Coefficient (odds)	z-value	p-value
(Intercept)		0.23	-0.53	0.6
bats	number of bats in roost	1	-1.2	0.23
days	days elapsed between the first case exposure and roost sampling	0.91	-1.3	0.2
distance	distance (km) between the case house and the roost	0.87	-0.74	0.46
cases	number of human spillover cases associated with each sampled roost	109	1.8	0.075

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467 **Appendix Table 3.** Model selection for variables associated with the presence of Nipah virus
468 RNA at *Pteropus medius* roosts (n = 22) near human cases.

Model	df	AICc	ΔAICc
(Intercept)	1	27.98	0
cases	2	28.01	0.03
bats + cases	3	28.33	0.35
cases + days	3	28.78	0.8
days	2	29.02	1.04
bats + cases + days	4	29.5	1.51
bats	2	30.03	2.05
distance	2	30.4	2.42
cases + distance	3	30.67	2.69
bats + cases + distance	4	31.17	3.19
bats + days	3	31.26	3.28
cases + days + distance	4	31.5	3.57
days + distance	3	31.71	3.73
bats + cases + days + distance	5	32.3	4.32
bats + distance	3	32.73	4.75
bats + days + distance	4	34.16	6.18

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470 **Appendix Table 4.** Changes in the proportion of urine aliquots testing positive over repeated
471 visits and associated cycle threshold (CT) values from RT-qPCR.

Roost	Sampling date	Urine aliquots positive	Average CT value
2013 Manikganj 9	11 April 2013	15/25 (60%)	30.5
	23 April 2013	3/30 (10%)	36.8
	6 May 2013	0/20 (0%)	ND
2013 Rajashahi 12	1 February 2013	2/20 (10%)	38.6
	9 February 2013	0/50 (0%)	ND

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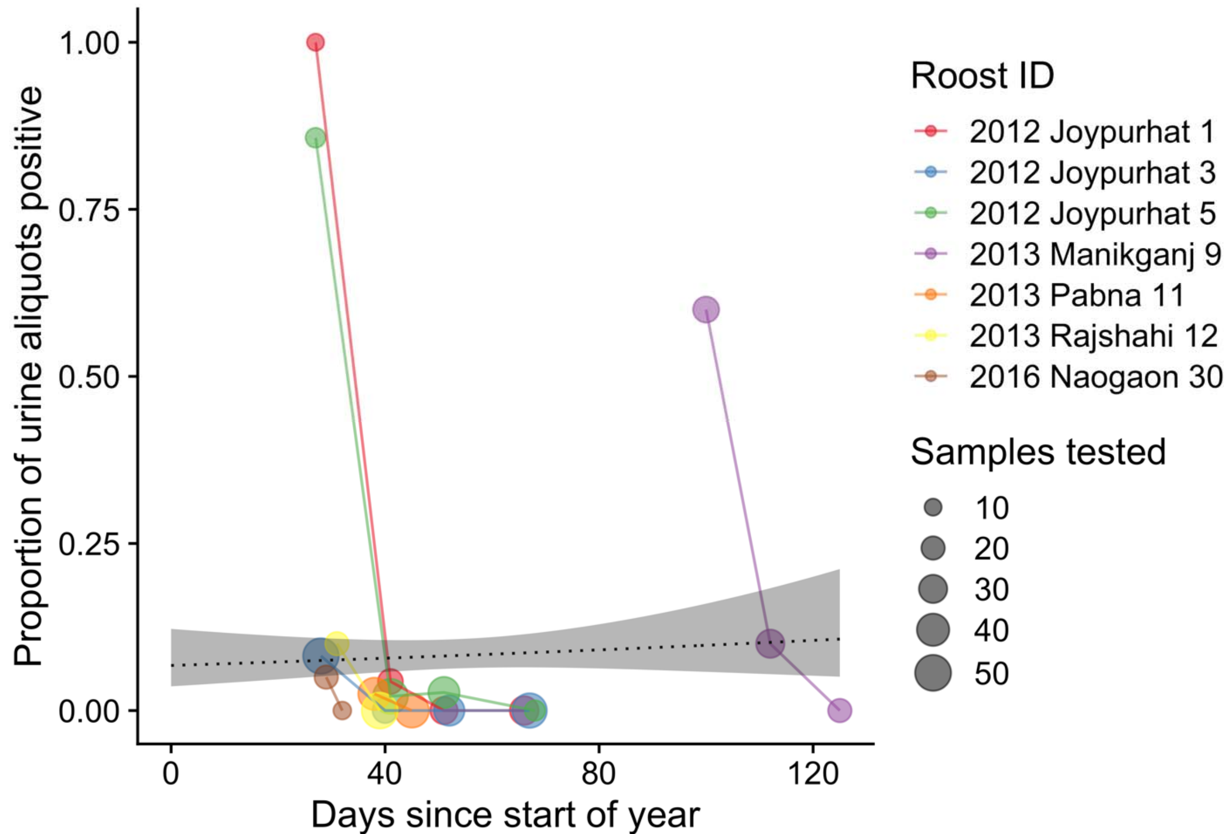

473

474 **Appendix Figure 1.** Results of screening *Pteropus medius* roost urine aliquots for Nipah virus
475 RNA. For each roost, the proportion of urine aliquots out of the total tested (shown by the size of
476 points) is aligned along a time axis of the days since the start of the calendar year for each roost
477 investigation.

478 **Appendix Dataset 1.** Data on the location of roosts, testing dates, and status of human cases.

479 **Appendix Dataset 2.** Compilation of publicly available Nipah virus sequences obtained from
480 bats and human cases.