1   **Ecological conditions experienced by bat reservoir hosts predict the intensity of Hendra**

2   **virus excretion over space and time**

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13   **Running head**: Ecological drivers of Hendra virus shedding

14   **Keywords:** bats; nutritional stress; pathogen spillover; generalized additive models

Abstract

The ecological conditions experienced by wildlife reservoir hosts affect the amount of pathogen they excrete into the environment. This then shapes pathogen pressure, the amount of pathogen available to recipient hosts over space and time, which affects spillover risk. Few systems have data on both long-term ecological conditions and pathogen pressure, yet such data are critical for advancing our mechanistic understanding of ecological drivers of spillover risk. To identify these ecological drivers, we here reanalyze shedding data from a spatially replicated, multi-year study of Hendra virus excretion from Australian flying foxes in light of 25 years of long-term data on changing ecology of the bat reservoir hosts. Using generalized additive mixed models, we show that winter virus shedding pulses, previously considered idiosyncratic, are most pronounced after recent food shortages and in bat populations that have been displaced to novel habitats. We next derive the area under each annual shedding curve (representing cumulative virus excretion) and show that pathogen pressure is also affected by the ecological conditions experienced by bat populations. Finally, we illustrate that pathogen pressure positively predicts observed spillover frequency. Our study suggests that recent ecological conditions of flying fox hosts are shifting the timing, magnitude, and cumulative intensity of Hendra virus shedding in ways that shape the landscape of spillover risk. This work provides a mechanistic approach to understanding and estimating risk of spillover from reservoir hosts in complex ecological systems and emphasizes the importance of host ecological context in identifying the determinants of pathogen shedding.

Introduction

Cross-species transmission can be conceptualized as a series of hierarchical barriers that must be overcome for a pathogen to move from a reservoir host to recipient hosts such as humans [1]. At the beginning of this cascade, the ecological conditions experienced by reservoir hosts shape susceptibility to infection and the likelihood of pathogen shedding (i.e., excretion) [2,3]. For example, droughts have been associated with increased nematode shedding in ungulates [4], and *Salmonella* shedding by ibis is more likely in urban than rural habitats [5]. This in turn determines pathogen pressure, or the amount of pathogen available to recipient hosts over space and time, which affects the force of infection and the ultimate probability of spillover [6]. Identifying the ecological conditions that drive pathogen pressure could accordingly improve reservoir host surveillance efforts and help predict where and when spillover is most likely [7,8].

Multiple challenges restrict our ability to establish the ecological drivers of pathogen pressure. On the one hand, many host populations must be repeatedly sampled to capture spatial and temporal variation in shedding [9]. This presents logistical difficulties and often necessitates optimized allocation of sampling effort [10,11]. On the other hand, the complex ecological dynamics used as predictors are best characterized with long-term research [12,13]. For example, more than two decades of annual monitoring were necessary to identify the ecological conditions that predict the density of infected nymphal ticks and human Lyme disease risk [14]. As such, the general sparsity of long-term, ecological data in many wildlife pathogen systems limits inference about the possible factors that shape pathogen pressure from reservoir hosts. Systems with data on pathogen pressure and long-term ecological conditions are critical for advancing our mechanistic understanding of spillover and to identify likely drivers in less studied systems.

56   Hendra virus (HeV) shedding from Australian flying foxes (*Pteropus* spp.) provides an

57   ideal system to identify the ecological conditions that predict pathogen pressure [15]. HeV

58   emerged in 1994, causing an outbreak of a lethal disease in horses and then humans that has been

59   followed by over 60 spillovers through 2019 across eastern Australia [16,17]. Flying foxes are

60   the natural reservoirs of HeV [18], and transmission to horses likely occurs via contact with

61   pasture, feed, or water contaminated with urine, saliva, or feces [19]. Spatiotemporal sampling of

62   bat roosts has revealed shedding pulses during the Austral winter in subtropical regions, yet their

63   occurrence varies both spatially and interannually [20]. This suggests that seasonal processes

64   such as births and climate shifts cannot solely drive pathogen pressure [21]. The mechanisms

65   underlying shedding pulses remain poorly understood but have been hypothesized to stem from

66   synchronous ecological conditions that affect bat behavior and within-host processes [22–25].

67   Traditionally, flying foxes move nomadically to find ephemeral sources of nectar in

68   native forests [26,27]. However, few dietary plants reliably flower in the Austral winter [27,28].

69   These plants occur in forests that have been mostly cleared for agriculture and urbanization,

70   making native winter feeding habitat scarcer and more dispersed [29]. Eucalypt flowering is

71   especially vulnerable to disruption from temperature and rainfall variability [28,30], and

72   occasionally winter or spring nectar fails, leading to acute food shortages and periods of

73   nutritional stress [27,31,32]. Bats have responded to these changes by splitting into smaller

74   roosts that are closer to reliable, often introduced, food in agricultural areas and urban gardens

75   [27,33]. Bats in these newly established and continually occupied roosts supplement their diet

76   with non-native plants [34,35]. This change in behavior has expanded the winter distribution of

77   flying foxes in subtropical Australia into areas that do not provide native winter food [36,37].

78   Reliance on agricultural and urban dietary resources when native plants are unavailable

79   could combine with other energetic stressors, such as seasonal reproduction and the more

80   extreme temperatures that bats can experience in these newly established roosts outside their

81   historic wintering range [38–40]. These stressors could alter bat within-host dynamics (e.g.,

82   immunosuppression) in ways that increase susceptibility to HeV or facilitate reactivation from

83   chronic infections [22,24,25,41]. Periods of nutritional stress have been associated with poor

84   body condition and elevated HeV seroprevalence in little red flying foxes (*P. scapulatus*) [31];

85   moreover, poor condition was a correlate of HeV infection in black flying foxes (*P. alecto*) but

86   was negatively correlated with HeV seroprevalence in *P. alecto* and grey-headed flying foxes (*P.*

87   *poliocephalus*) [42,43]. The relationships between food shortages and HeV shedding therefore

88   remain poorly understood, especially in the context of the changing ecological conditions

89   experienced by these bat hosts. Recent work suggests such ecological changes have increased the

90   frequency of HeV spillovers (Eby et al., in review) [17,44], but the mechanisms linking food

91   shortages and dramatic shifts in bat behavior with virus shedding dynamics remain unresolved.

92   Here, we reanalyze shedding data from a spatially replicated, multi-year study of HeV in

93   flying foxes in light of long-term data on bat ecology to test whether food shortages and recent

94   shifts in flying fox behavior amplify pathogen pressure. We first ask whether these ecological

95   conditions can explain seemingly idiosyncratic seasonal shedding pulses while also accounting

96   for underlying spatiotemporal variation in infection dynamics and roost composition, including

97   species with high competence (e.g., *P. alecto*) [45–47]. We next quantify pathogen pressure per

98   year and per roost and test whether bat ecology also predicts cumulative seasonal shedding

99   intensity across the landscape. Finally, we assess the relationship between pathogen pressure and

100   the frequency of realized spillover events. Our work thus provides a mechanistic approach to

101   understanding bat-borne virus spillover risk as well as a new method for estimating these risks

102   from multiple ecological predictors. Bats throughout the eastern hemisphere are experiencing

103   similar stressors owing to land conversion [23,48], underscoring the general need to better

104   understand the likely consequences for pathogen shedding and subsequent spillover risk.

105

106   **Materials and methods**

107   *Spatiotemporal data on HeV shedding*

108   We reanalyzed a previously published longitudinal dataset of HeV shedding from flying foxes

109   spanning July 2011 to November 2014 across New South Wales and Queensland [20]. Sampling

110   consisted of urine collection from quadrants of 3.6 m x 2.6 m plastic sheets placed under roosts

111   at primarily monthly intervals [49]. Urine was pooled per quadrant and screened by quantitative

112   RT-PCR targeting the HeV M gene to determine presence or absence of viral shedding in a roost

113   [50]. We aggregated data to the scale of week and determined HeV prevalence as the proportion

114   of positive urine pools. Similarly, we calculated the median number of all flying foxes and for

115   each Australian *Pteropus* species per roost per time interval. We restricted HeV data to an area

116   of subtropical eastern Australia (i.e., mid-to-northeast New South Wales, southeast Queensland)

117   that represents our study region for long-term data on bat ecology (see below) and includes the

118   locations of almost all subtropical HeV spillovers [15]. We further restricted analyses to 2012

119   through 2014, as 2011 sampling mostly occurred at a single roost [20]. We also limited analyses

120   to roosts sampled in at least two of the three years and in at least three timepoints per year. We

121   thus analyzed prevalence data from nine roosts sampled in 2012–2014 (*n*=196 weeks; Fig. 1).

122

123   *Roost-level ecological conditions*

124   We paired HeV prevalence data within our study region with long-term data on flying fox

125   behavior. As described in full in Eby et al. (in review), we used data held by Australian state

126   governments, flying fox surveys conducted between 1998–2005 and 2012–2019, records held by

127   local land managers and experienced observers, and direct observation by the authors to

128   characterize the ecological conditions experienced by bat populations over time [27,28,37].

129   Roosts were classified as belonging to their historic winter range (and thus where bats rely on

130   native winter dietary plants) or to newly established overwintering regions (and thus where

131   native food is unavailable). We also used these data to identify regional food shortages, acute

132   periods associated with reduced flowering of native dietary plants or no flowering (Table 1).

133

134   *Analyses of HeV shedding*

135   To test if roost type (i.e., historic or new overwintering regions) and regional food shortages

136   affect seasonal HeV shedding, we fit generalized additive mixed models (GAMMs) with a

137   binomial response to urine pool prevalence with the *mgcv* package in R [51,52]. GAMMs can

138   flexibly approximate the temporal dynamics of infectious diseases, even when underlying

139   transmission mechanisms are unknown [53,54]. Our fixed effects included a cyclic cubic spline

140   for week, ordered factors of roost type and regional food shortage, and all possible interactions.

141   We used regional food shortages from the prior year (i.e., October 2012 and October–November

142   2013; Fig. 1) owing to previously observed associations between spring and summer food

143   shortages and subsequent winter spillovers of HeV in 2010–2011 and in 2016–2017 [44].

144   We also adjusted for occupancy of *P. alecto* using its relative abundance per roost from

145   weekly median flying fox counts. We did not account for co-roosting *P. poliocephalus* and *P.*

146   *scapulatus*, as *P. alecto* is the only species that has been associated with HeV shedding in our

147   study region [45,46]. As flying foxes roost in aggregations of up to hundreds of thousands of

148   individuals, counts and resulting species proportions are estimates. We thus derived a binary

149   variable denoting if the weekly proportion of *P. alecto* per roost was below or above 25%, the

150   lowest maximum proportion of this species across all roosts in this dataset (Fig. S1) [20]. This

151   cutoff accordingly indicates the upper occupancy bound of this species when it is rare. Our

152   GAMMs also controlled for spatial dependence through a bivariate smooth of longitude and

153   latitude [55]. To account for residual variation in spatiotemporal shedding, we included a random

154   factor smooth of week per roost per year [56]. We set the random effect such that all groups can

155   differ in weekly shedding but are penalized if they deviate too strongly from the global trend.

156   Given the complexity of our GAMM relative to our sample size, we used an information

157   theoretic approach to select among competing nested fixed effects. We considered (*i*) our full

158   model alongside two simplified GAMMs: (*ii*) without the three-way interaction between week,

159   roost type, and prior year food shortage and (*iii*) without the two-way interaction between the

160   latter categorical predictors. We fit GAMMs with maximum likelihood (ML) and compared

161   models using corrected Akaike information criterion (AICc) and Akaike weights (w_i). We

162   considered models within two ΔAICc to be competitive [57]. We then refit our most supported

163   models with restricted ML (REML) for parameter estimation and visualizing fitted values.

164   To quantify cumulative annual shedding intensity, we fit univariate GAMs with binomial

165   response and cyclic cubic splines for week to our HeV prevalence data per year for each of our

166   nine roosts (*n*=25; Fig. S2). In rare cases with few (e.g., *n*=3) sampling events and where

167   prevalence was uniformly zero (i.e., as occurred in two annual time series; Fig S2), we used thin

168   plate splines to improve convergence. We then calculated the area under each annual shedding

169   curve (i.e., pathogen pressure; AUC) by integrating the fitted values and confidence intervals

170   using the *Bolstad2* package [53,58]. This measure summarizes the magnitude and duration of

171   shedding in a single metric, and thus greater pathogen pressure suggests more cumulative HeV-

172   positive pools per roost per year. We used the *metafor* package to assess heterogeneity in AUC

173   through a random-effects model weighted by sampling variance, which we derived from the

174   number of sampling events per time series [59]. We then used REML to quantify I^2, which

175   measures the contribution of true heterogeneity rather than noise to the variance in AUC [60].

176   We then assessed the ecological drivers of pathogen pressure using another set of GAMs.

177   We modeled annual AUC as a Tweedie-distributed response and again weighted these estimates

178   by the number of sampling events per annual time series. We included roost type and prior food

179   shortage as categorical predictors alongside a bivariate smooth of longitude and latitude. We also

180   controlled for the mean annual proportion of *P. alecto* per roost with a thin plate spline and

181   assessed sensitivity of our results to two alternative measures of annual relative abundance (e.g.,

182   median, winter maximum). Lastly, because a small number of our annual time series had short

183   sampling durations that did not include the Austral winter (Fig. S2), we reran our GAMs after

184   excluding four annual AUC estimates derived from 20 or fewer weeks of flying fox sampling.

185

186   *HeV spillover analyses*

187   To assess relationships between pathogen pressure and spillover, we collated data on the location

188   and date of all known HeV cases in horses in our study region between 2012–2014 [15]. As

189   described in full in Eby et al. (in review), these data were obtained from government notices (i.e.,

190   New South Wales Department of Primary Industries, Queensland Department of Agriculture and

191   Fisheries; Business Queensland), ProMED, local media reports, and personal communications.

192   We aggregated all HeV case locations to the nearest town or regional center for confidentiality.

193   We derived spatial buffers using the *rgeos* package to collate the total number of HeV

194   spillover events near each of our nine roosts per sampled year. Australian *Pteropus* are highly

195   mobile [32,61], and prior analyses have shown weak spatial synchrony in HeV shedding among

196   flying fox roosts up to approximately 500 km [21]. We therefore used spatial buffers of 50, 100,

197   200, 300, 400, and 500 km. We modeled counts of spillovers within each buffer through a set of

198   GAMs that used Poisson distributions and penalized splines for pathogen pressure [62]. As in

199   our GAM analyses of AUC, we also included a bivariate smooth of longitude and latitude.

200

201   **Results**

202   *Ecological predictors of seasonal HeV shedding pulses*

203   Our GAMMs revealed that previously observed seasonality in HeV shedding [e.g., 26] is

204   markedly explained by recent changes in bat behavior and by food shortages. The most

205   parsimonious model explained 71% of the deviance and only included interactions between week

206   and both roost type and recent food shortages (w_i=0.59; Table S1). Ordered factor smooths

207   indicated that roosts in newly established overwintering regions had distinct seasonal shedding

208   patterns compared to roosts in the historic wintering range (χ^2=23.61, p<0.001); roosts that

209   experienced a recent food shortage also displayed distinct shedding seasonality compared to

210   roosts without recent food shortages (χ^2=14.33, p<0.001; Table 2). In the absence of recent food

211   scarcity, historic roosts had negligible seasonality in shedding, while new overwintering roosts

212   exhibited shedding pulses in winter (Fig. 2A). Yet when food shortages occurred in the spring

213   prior to sampling (Fig. 2B), both historic and new overwintering roosts displayed stronger

214   shedding seasonality and amplitude the following winter. Further, the winter shedding amplitude

215   for new roosts was greater than historic roosts. Such patterns held after accounting for relative

216   *Pteropus alecto* occupancy (Fig. 2C–D), which positively predicted HeV prevalence (Table 2).

217

218   *Heterogeneity in pathogen pressure*

219   Cumulative HeV pathogen pressure (i.e., area under each annual shedding curve) varied

220   substantially across roosts and years (Fig. 3A). Our meta-analysis identified significant

221   heterogeneity among AUC estimates (I^2=0.93, Q_{24}=339.59, p<0.001). When testing the

222   ecological drivers of this variation, our GAM explained 40% of the deviance in pathogen

223   pressure (Table 3). Whereas recent food shortages had little effect on AUC (β=0.15, t=1.38,

224   p=0.19), newly established overwintering roosts had significantly greater pathogen pressure than

225   historic roosts (β=0.76, t=4.33, p<0.001; Fig 3B). Similar results were obtained when adjusting

226   for median annual and maximum winter fractions of *P. alecto* per roost (Table S2 & S3). In our

227   primary model, the mean annual proportions of *P. alecto* also significantly and largely positively

228   predicted AUC (F=7.88, p<0.001); similar results were obtained with median annual proportions

229   of *P. alecto* but not winter maximum occupancy (Fig. S3; Table S2 & S3). Lastly, excluding the

230   four AUC estimates from truncated time series mostly strengthened the associations between

231   predictors (roost type, food shortage, *P. alecto*) and pathogen pressure (Fig. S4; Table S4–6).

232

233   *Pathogen pressure and HeV spillover*

234   Within the period of flying fox sampling (2012–2014), 12 spillover events occurred within 500

235   km of our nine study roosts (Fig. 4A, Table S7). When aggregating spillovers within 50 km of

236   each roost, our GAM explained 53% of the deviance and was primarily driven by spatial

237   patterns, as AUC had a weak, nonlinear relationship with spillover (χ^2=4.74, p=0.11). However,

238   when expanding the buffers between 100 and 500 km from each roost, our GAMs explained 29–

239   45% of the deviance and predicted stronger associations between AUC and spillover (Table S8–

240   S13). Generally, GAMs suggested a generally positive relationship between pathogen pressure

241   and spillovers that was either maximized or saturated at intermediate-to-high AUC (Fig. 4B).

242

243   **Discussion**

244   Mechanistic understanding of cross-species transmission risk remains hindered by limited

245   information on the relationships between reservoir host ecology, pathogen pressure, and

246   observed spillover events [1,6,7,15]. By combining data on long-term ecology with the spatial

247   and temporal distribution of infection, we here demonstrate that the ecological conditions

248   experienced by reservoir hosts shape pathogen shedding. Prior work has shown that pulses of

249   HeV excretion from flying foxes often occur in the Austral winter and that drier conditions in

250   prior seasons are associated with peak annual shedding [20,21]. However, contextualizing these

251   seasonal and climatic patterns with bat ecology here reveals that these periods of intensive virus

252   shedding predominantly occur after food shortages and in new overwintering roosts in urban and

253   agricultural habitats. By quantifying an aggregate metric of pathogen pressure, we also show that

254   the accumulated intensity of HeV shedding provides distinct insights into these ecological

255   drivers, as pathogen pressure is governed more by whether the roost is located in regions with

256   native winter resources than by periods of recent food scarcity. Lastly, pathogen pressure was

257   positively associated with cases of HeV in horses at a regional scale, which provides support for

258   the spatiotemporal intensity of virus shedding as a mechanistic determinant of spillover risk.

259   Foremost, our findings emphasize that the ecological conditions experienced by reservoir

260   hosts predict pronounced pulses of virus shedding and overall pathogen pressure. Across eastern

261   Australia, habitats for the winter-flowering plants that drive flying fox nomadism have been

262   reduced through land clearance, in turn driving nutritionally stressed bats into novel urban and

263   agricultural environments outside their typical overwintering range (Eby et al., in review)

264   [27,32,36,37]. Prior work has proposed, but not tested, that HeV shedding from flying foxes is

265   driven by this process [23–25,63]. Acute food shortages could produce cumulative effects with

266   other energetic and seasonal stressors (e.g., pregnancy, thermoregulation), as well as reliance on

267   poor-quality, non-native food in agricultural and urban habitats, to alter within-host dynamics of

268   viruses in bats [22,23]. Prior work has suggested that the physiological demands of winter

269   thermoregulation in parts of the *P. alecto* range could drive observed negative correlations

270   between minimum temperature and urinary cortisol excretion [40]. Our results support the idea

271   of cumulative stressors, as HeV shedding was greatest not only in winter but also in roosts that

272   were established in novel overwintering habitats and after a food shortage in the prior spring.

273   Increased viral shedding associated with suboptimal habitats or food shortages could be

274   driven by immunosuppression, such that cumulative stressors may impair HeV tolerance and

275   facilitate replication or allow latent infections to reactivate [25,64]. Recrudescent infection of

276   henipaviruses in bats has been proposed to explain shedding pulses that coincide with stressors

277   [24,25] and for seroconversions in captivity [65]. The mechanism driving the time lag between

278   acute food shortages, which typically occur in spring, and increased HeV shedding in the

279   following winter (i.e., in the subsequent 6–9 months; Fig. 1) remain unknown; however, the

280   cumulative impact of multiple stressors (e.g., long-term nutritional stress, cold temperature,

281   pregnancy) offers a potential explanation. Future studies, including immune assessments of wild

282   bats and factorial experiments, will be critical to mechanistically understand the causal effect of

283   spring food shortages, bat residency outside their winter range, dietary reliance on non-native

284   plants, and other energetic stressors (e.g., pregnancy) on subsequent winter pulses of HeV

285   shedding. Such work would also provide foundational insights into how bats control zoonotic

286   viruses more broadly, given likely effects of stressors on immunity and tolerance [66,67].

287   Pathogen pressure has been suggested to be a critical determinant of spillover risk in

288   recipient hosts [1,6], but quantifying the spatial and temporal distribution of shedding in ways

289   that facilitate downstream analyses has remained challenging. Here, we propose the area under

290   annual shedding curves (i.e., AUC) as a meaningful metric to summarize pathogen output into

291   the environment. AUC has previously been used to summarize the cumulative pathogen output

292   from within-host dynamics (e.g., *Mycoplasma agassizii* intensity across individual tortoises [68])

293   and number of infected individuals (e.g., fungal infections across *Daphnia* populations [69,70]),

294   which capture host infectiousness and local epidemic size, respectively, rather than the explicit

295   infectious dose available to recipient hosts. For the latter, epidemic size has in turn been linked to

296   local ecological conditions, such as lake temperature and host community composition [69,70];

297   however, such metrics have not been applied to replicate reservoir host populations to identify

298   predictors of pathogen pressure. In one case study of a zoonotic pathogen reservoir, data on

299   avian influenza virus prevalence in ducks were used to derive AUC across three regions of North

300   America, but inference on its underlying ecological drivers was limited by few replicates [53].

301   In our analyses, AUC not only displayed substantial heterogeneity, but also varied with

302   roost ecology in ways that differed from seasonal GAMMs. Whereas the timing and amplitude of

303   shedding was driven both by roost type and prior food shortages, AUC was exclusively driven by

304   roost type. This contrast could relate to the tension between a short but intense shedding pulse

305   and a smoldering shedding dynamic with lower intensity. Short but intense shedding pulses

306   observed after food shortages could produce similar AUC to the lower amplitude shedding that

307   occurred year-round in roosts without acute food scarcity. Such results emphasize how pathogen

308   pressure can offer distinct insights into infection dynamics relative to shedding seasonality alone.

309   High pathogen pressure should theoretically have a pronounced effect on shaping the

310   force of infection and the ultimate probability of spillover, given the hierarchical nature of cross-

311   species transmission [1,6]. However, empirical support for pathogen pressure from reservoir

312   hosts influencing disease cases in recipient hosts has been restricted by the rarity of spillover in

313   many systems, such that evidence is mostly from temporal overlap between shedding events and

314   outbreaks or broad-scale, regional analyses of reservoir infection and human disease data. For

315   example, Marburg virus spillover to humans coincide with seasonal birth pulses of Egyptian fruit

316   bats, when host shedding is most pronounced [71], and the density of bank voles seropositive for

317   Puumala virus can also explain regional human incidence of nephropathia epidemica [72].

318   The relatively high frequency of HeV spillovers compared to less-frequent spillover in

319   many other systems provides a tractable system for linking pathogen pressure and cross-species

320   transmission [73]. Our analyses show that pathogen pressure (i.e., annual AUC) positively

321   predicts observed spillovers within the broad, regional scales of flying fox movements (i.e., up to

322   500 km). As a caveat, no spillovers were recorded within typical nightly foraging ranges (i.e., 25

323   km) of the roosts monitored in our study region during the period of HeV surveillance [26,32].

324   Our findings therefore assume that the ecological conditions experienced by unsampled roosts in

325   closer proximity to spillover events may be similar to those of our sampled roosts up to 500 km.

326   As our patterns were consistent across a broad span of spatial scales relevant to flying fox

327   mobility, our results suggest that the association between AUC and spillover are likely more

328   affected by interannual variation in pathogen pressure, such as that driven by acute food

329   shortages. Associations between pathogen pressure and spillovers were also mostly positive and

330   monotonic but generally not exclusively linear. Importantly, our measure of pathogen pressure

331   accumulates processes in the spillover hierarchy—including reservoir host distribution, reservoir

332   host density, infection prevalence, and infection intensity—up to and including shedding into the

333   environment [1]. Downstream factors such as HeV survival in the environment and recipient host

334   distribution and susceptibility may moderate effects of high pathogen pressure on spillover risk

335   [6,74]. Additionally, short but high amplitude pulses of virus shedding, such as those observed in

336   the year following acute food shortages, may ultimately provide a more sufficient HeV dose to

337   horses, thus resulting in roughly equivalent spillover risks from moderate and high AUC [1,75].

338   Because summarizing the spatiotemporal intensity of infection both provided distinct

339   insights into ecological drivers of pathogen shedding and predicted spillover at broad regional

340   scales, we suggest quantifying pathogen pressure would be particularly tractable and useful in

341   systems where recipient host exposure occurs following pathogen release into the environment.

342   Such systems include but are not limited to bat filoviruses and coronaviruses, avian influenza

343   viruses, and helminths, protozoa, and fecal-oral bacteria of various wildlife [4,71,76,77]. Careful

344   sampling designs for these environmentally shed pathogens, such as under-roost sampling

345   methods for bats, could facilitate estimating pathogen pressure and, in turn, spillover risks [78].

346   In conclusion, we show that the ecological conditions experienced by bat reservoir hosts

347   shape the timing, magnitude, and cumulative intensity of zoonotic virus shedding in ways that

348   subsequently predict observed spillover events. Importantly, such inferences were only possible

349   by integrating both spatiotemporal data on infection with long-term studies of host ecology and

350   behavior, which demonstrates the importance of long-term, spatially replicated studies of

351   reservoir hosts for linking environmental change and zoonotic pathogen dynamics. Despite a

352   long-standing recognition of spatial and temporal scale in ecological research [79], replication

353   across both axes remains challenging, particularly over relevant time intervals [80].

354   Spatiotemporal sampling is especially critical in the study of infectious disease [9,11], because

355   pathogen shedding and transmission are both inherently spatial and temporal processes [63,81].

356   Connecting such data with changing ecology of wildlife further requires studies of abiotic and

357   biotic correlates and host behavior and demography at similar or biologically meaningful spatial

358   and temporal scales [7,14,82]. Here, the ecological conditions that predict HeV shedding from

359   flying foxes were derived from behavioral data collected over approximately 25 years and from

360   diverse sources [27,28,37] (Eby et al., in review). Although collecting this kind of ecological

361   data will accordingly present logistical difficulties, the growth of national research networks,

362   global community consortiums, and remote sensing, among other large-scale efforts, should

363   facilitate similar approaches to link spatiotemporal data on both host ecology and infection [83].

364   In turn, careful attention to the ecology of reservoir hosts through long-term monitoring could

365   facilitate improved early detection and preemptive management of zoonotic spillover events.

366

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379

380   **Author contributions**

381   DJB, PE, AJP, and RKP designed the study; PE, AJP, WM, and RKP collected, collated, and

382   managed data; DJB analyzed data; and DJB wrote the manuscript with feedback from coauthors.

383

384   **Data availability**

385   All HeV shedding data and flying fox data used in this analysis are freely available from the

386   Queensland government (CC BY 4.0; https://www.data.qld.gov.au/dataset/hev-infection-flying-

387   foxes-eastern-australia) [20]. Ecological covariates of study roosts are provided in Table 1. Data

388   on HeV spillovers within 500 km of our study roosts between 2012–2014 are provided to the

389   nearest town in Table S4; finer-scale data are confidential as they identify individual properties.

390

391   **Code availability**

392   Code for estimating AUC from each annual time series per roost will be provided in the

393   Supplemental Material upon acceptance.

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617

618 **Tables**

619 Table 1. Spatiotemporal ecological conditions of flying fox roosts included in the analysis.

Roost	Longitude	Latitude	Year	Food shortage in prior year	Roost type
Toowoomba	151.954	-27.564	2012	0	New overwintering
			2013	0	
Boonah	152.681	-27.992	2012	0	New overwintering
			2013	0	
			2014	1	
Alstonville	153.439	-28.481	2013	0	Historic
			2014	1	
Lismore	153.277	-28.807	2012	0	Historic
			2013	0	
			2014	1	
Bellingen	152.897	-30.452	2012	0	Historic
			2013	1	
			2014	1	
Nambucca Heads	153.003	-30.642	2012	0	New overwintering
			2013	1	
			2014	1	
Port Macquarie	152.908	-31.431	2012	0	Historic
			2013	1	
			2014	1	
Wingham	152.376	-31.871	2012	0	Historic
			2013	1	
			2014	1	
Singleton	151.175	-32.562	2012	0	New overwintering
			2013	1	
			2014	1	

620

621 Table 2. Results of the most parsimonious GAMM for predicting seasonal HeV shedding from

622 Australian flying foxes (*n*=196), fit using REML. Fixed effects are presented as ordered factors

623 with coefficients (categorical) or the estimated degrees of freedom (EDF) and test statistics.

Term	β	z	EDF	χ^2	p
Intercept	-3.94	-23.14			<0.001
New overwintering	-0.19	-0.79			0.43
Prior food shortage	-0.16	-0.70			0.49
Common *P. alecto*	0.56	3.17			0.002
s(week)			0.01	0.01	0.04
s(week) : new overwintering			4.12	23.61	<0.001
s(week) : prior food shortage			3.19	14.33	<0.001
s(longitude, latitude)			0.00	0.00	0.38

624

625 Table 3. Results from the GAM of annual AUC estimates from flying fox roosts (*n*=25), fit using

626 REML. Fixed effects are presented as ordered factors with model coefficients (categorical) or the

627 estimated degrees of freedom (EDF) and test statistics.

Term	β	t	EDF	F	p
Intercept	0.03	0.28			0.79
New overwintering	0.76	4.33			<0.001
Prior food shortage	0.15	1.38			0.19
s(mean percent *Pteropus alecto*)			3.30	7.88	<0.001
s(longitude, latitude)			3.37	4.56	<0.01

628

629   **Figures and legends**

630

631   Figure 1. Spatiotemporal variation in HeV shedding for the nine flying fox roosts. Curves display

632   the weekly proportion of HeV-positive urine pools, with roosts shown in order of latitude and

633   colored by roost type. Ticks show sampling timepoints. Grey shading indicates regional acute

634   food shortage events, and dashed lines indicate the Austral winter (i.e., June through August).
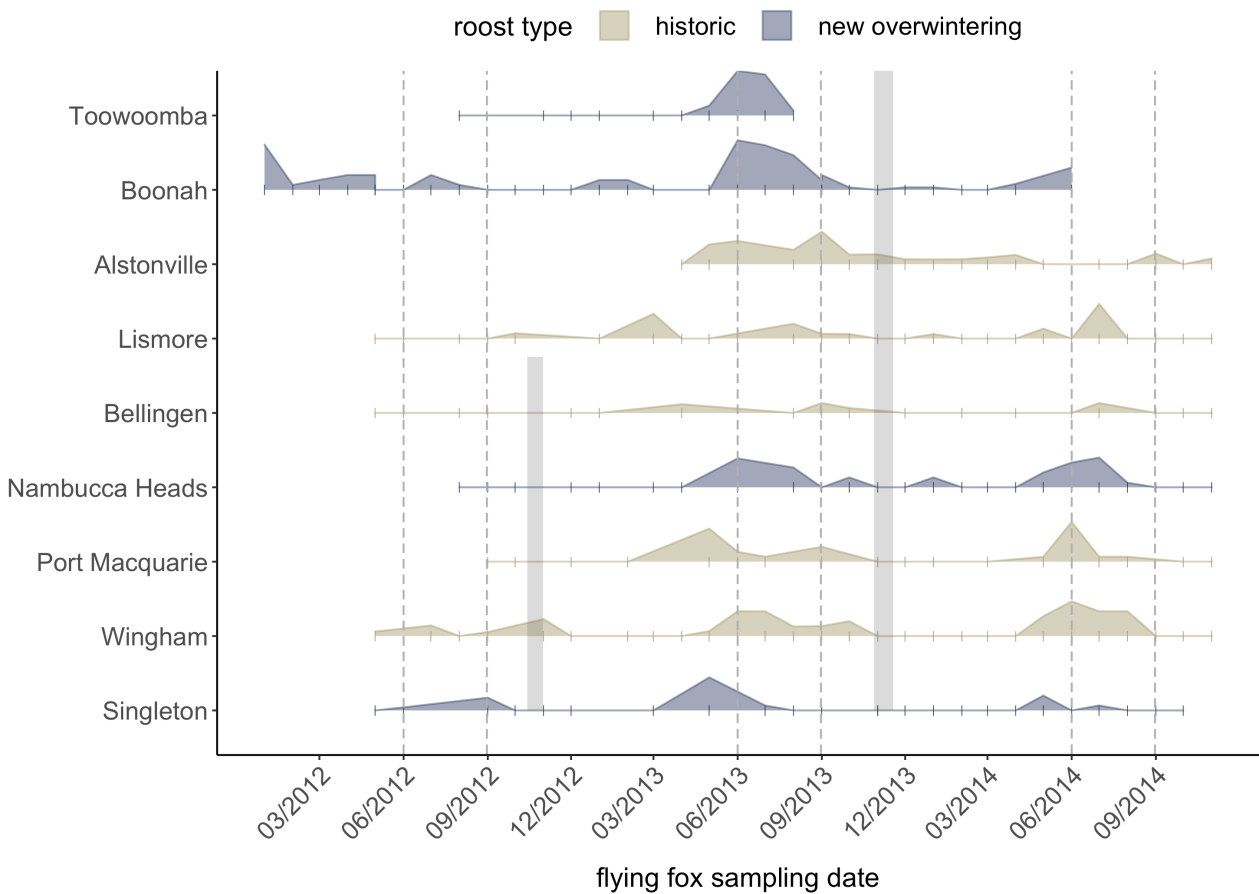

635

636   Figure 2. Fitted HeV urine pool prevalence and 95% confidence intervals from the most

637   parsimonious GAMM with week, seasonal interactions with roost type and previous food

638   shortages, the interaction between both factor variables, and an adjustment for categorical

639   weekly *Pteropus alecto* occupancy. Weekly data are overlaid and colored by roost type. Thin

640   lines show the fitted curves from the random factor smooth including each roost per year.
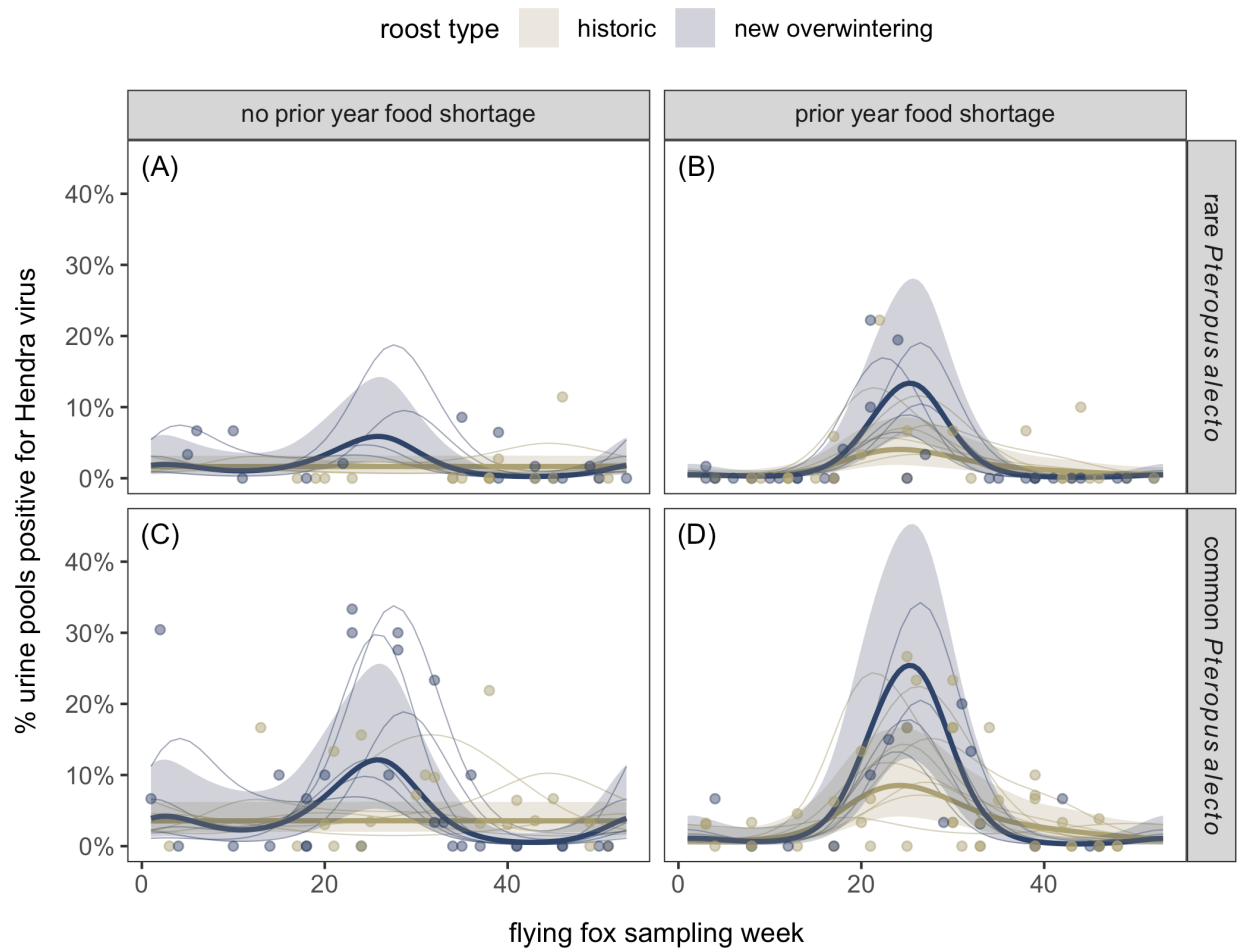

641

642   Figure 3. Variation in HeV pathogen pressure from flying foxes. The forest plot displays annual

643   AUC estimates and 95% confidence intervals ordered by latitude and year; points are scaled by

644   the number of sampling events per annual time series as weights. The horizontal axis uses a

645   modulus transformation to accommodate wide upper bounds of some confidence intervals. Right

646   displays fitted values and 95% confidence intervals for the GAM, with raw data (scaled by the

647   number of sampling events per each time series) and modeled means colored by roost type.

648   Transparency denotes AUC estimates derived from truncated annual time series ($\leq$ 20 weeks).
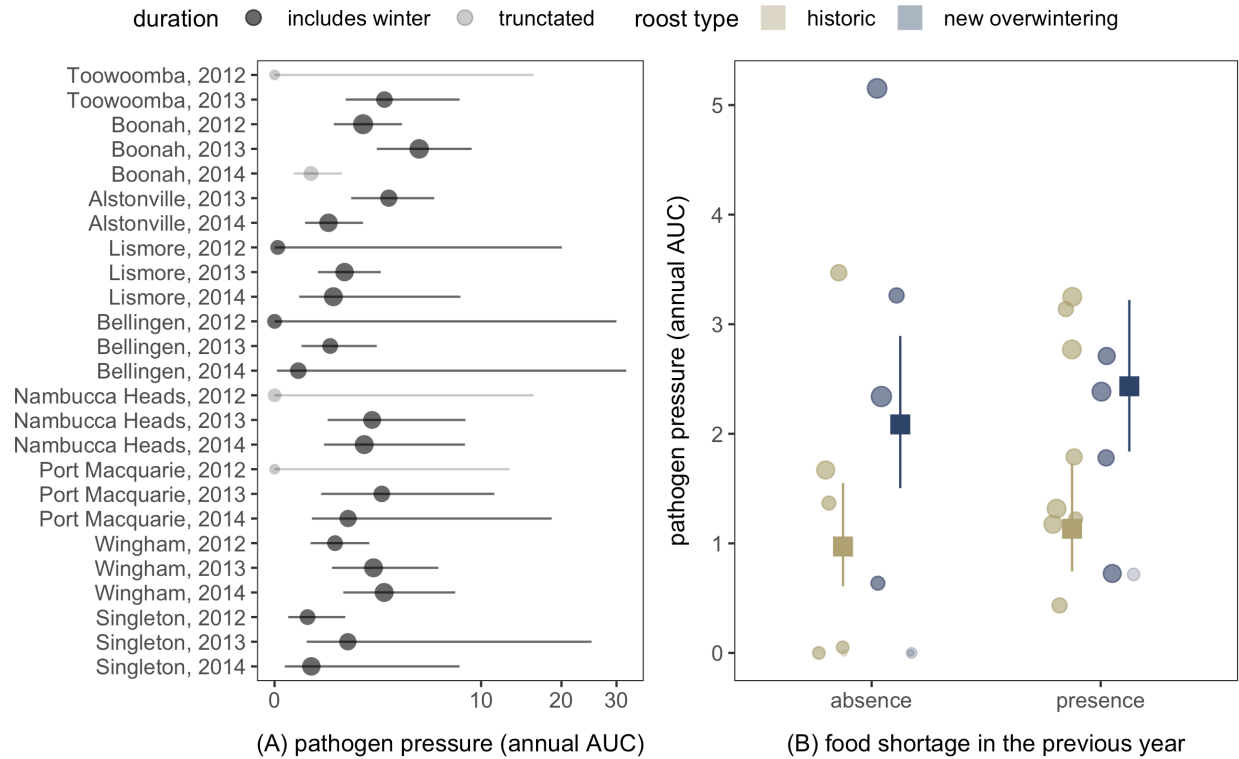

649

650   Figure 4. Spatiotemporal variation in regional HeV spillovers during the flying fox surveillance

651   period (2012–2014) and its relationship with pathogen pressure (AUC). (A) Maps display the

652   annual distributions of spillovers (colored by year) in relation to the nine analyzed roosts.

653   Modeled relationships between AUC and spillover counts are shown with fitted values and 95%

654   confidence intervals from GAMs for 50, 100, 200, 300, 400, and 500 km buffers of each roost.

655