

1 **Manipulating vector transmission reveals local processes in bacterial**
2 **communities of bats**

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22

23 **Abstract**

24 Infectious diseases result from multiple interactions among microbes and hosts, but
25 community ecology approaches are rarely applied. Manipulation of vector populations provides a
26 unique opportunity to test the importance of vectors in infection cycles while also observing
27 changes in pathogen community diversity and species interactions. Yet for many vector-borne
28 infections in wildlife, a biological vector has not been experimentally verified and few
29 manipulative studies have been performed. Using a captive colony of fruit bats in Ghana, we
30 observed changes in the community of *Bartonella* bacteria over time after the decline and
31 subsequent reintroduction of bat flies. With reduced transmission, community changes were
32 attributed to ecological drift and potential selection through interspecies competition mediated by
33 host immunity. This work demonstrated that forces maintaining diversity in communities of free-
34 living macroorganisms act in similar ways in communities of symbiotic microorganisms, both
35 within and among hosts. Additionally, this study is the first to experimentally test the role of bat
36 flies as vectors of *Bartonella* species.

37

38 **Keywords:** *Bartonella*; bats; ecological dynamics; pathogen diversity; vector-borne bacteria;
39 community assembly

40

41 **Introduction**

42 Knowledge of the processes driving parasite diversity is central to understanding infection
43 dynamics in endemic populations and pathogen emergence in new hosts. In contrast to an
44 historical focus on simple one-host, one-parasite systems, there is now greater appreciation that
45 parasites exist within communities of other parasites, harbored by hosts that may vary in their
46 responses to parasitism (Johnson et al., 2015). Yet it is not clear how well ecological theory
47 developed for free-living organisms applies to communities of microorganisms (Sutherland et al.,
48 2013). This is especially true for parasites and symbionts due to the environmental feedbacks that
49 exist from their dependence on hosts for survival and reproduction (Costello et al., 2012; Miller
50 et al., 2018). Additionally, parasite community dynamics within hosts may occur at differing
51 timescales compared to transmission among hosts. Given these differences, experimental
52 manipulations of natural parasite communities are needed to explore the generality of community
53 theory across organisms.

54 The metacommunity concept is a useful framework to apply toward analyzing parasite
55 community dynamics within hosts (Leibold et al., 2004; Mihaljevic, 2012). In this framework,
56 hosts are discrete patches harboring potentially interacting parasite species. Similar to free-living
57 organisms, four forces might be expected to affect parasite community diversity: speciation,
58 dispersal, ecological drift, and ecological selection (Vellend, 2010). Within a metacommunity,
59 the relative importance of these forces may vary at different scales (Seabloom et al., 2015), i.e.,
60 within versus among hosts. Speciation is the only force that generates parasite diversity *de novo*,
61 but is generally slow and dependent upon dispersal for newly created diversity to penetrate to all
62 scales. Dispersal is the movement of parasite species within a host, among hosts through
63 transmission, or among host populations through host movement. Within metacommunities,

64 parasite species with equal competitive ability may vary stochastically in the production of new
65 parasite individuals or in new infections through transmission. This ecological drift can lead to
66 changes in community composition within hosts (e.g., loss of rare species) or among hosts (e.g.,
67 increases in beta diversity), similar to predictions of neutral theory (Hubbell, 2001). Drift
68 happens faster in small communities with few parasite individuals and with little dispersal.
69 Lastly, ecological selection acts within and among hosts. Selection occurs because parasite
70 species vary in replication success within different host individuals or species because of
71 variation in susceptibility or tolerance. Additionally, parasite species may compete within a host,
72 either indirectly through shared resources or common enemies, such as the host immune system,
73 or directly through interference (Pedersen & Fenton, 2007). Species with higher success within a
74 host will dominate and may exclude others, but this can be counterbalanced if fitness is driven by
75 dispersal ability over interspecific competition or there is frequency-dependent selection by the
76 host immune system. These four forces could separately affect parasite community diversity over
77 time. While speciation ultimately creates diversity, the other forces sort parasite species across
78 scales. Thus, a strategy for studying parasite community diversity is to understand the relative
79 importance of these forces both within and among hosts (Seabloom et al., 2015).

80 Manipulative experiments are one approach to measuring the relative influence of
81 ecological forces acting on communities. By changing the strength of one force, one can observe
82 how others respond and interact across scales. While previous studies have performed parasite
83 community manipulations within and among hosts (see Mihaljevic, 2012 and Johnson et al., 2015
84 for examples), few studies to our knowledge have looked at how manipulating forces that act
85 across scales lead to changes in other forces. Since dispersal is the force that interacts with other

86 processes across within-host and among-host scales (Vellend, 2010), it is an appealing target for
87 manipulation.

88 Vector-borne infections are ideal systems for experimental study because reduction of
89 vector density limits dispersal of parasites between hosts, allowing the analysis of other forces
90 affecting the relative abundance of parasite species. Using a captive colony of straw-colored fruit
91 bats (*Eidolon helvum*) in Ghana, the community dynamics of *Bartonella* bacteria were monitored
92 in bats over three years. During this experiment, the presumed vectors (bat flies) declined in
93 density within the colony but were then reintroduced. The experiment thus controls parasite
94 dispersal across two scales: the captive colony is closed to immigration (pups enter the colony
95 uninfected) and transmission is manipulated via changes in the bat fly population size. By
96 manipulating parasite dispersal, the effect of among-host dispersal is minimized and the effects of
97 local, within-host effects (ecological drift and selection) on parasite dynamics and diversity can
98 be observed. We hypothesize that *Bartonella* communities in the colony will respond to changes
99 in among-host dispersal/transmission by bat flies. Specifically, we predict that infection
100 prevalence and diversity will at first decline concurrently with the bat fly population and then
101 increase upon reintroduction of flies, thus providing experimental evidence that bat flies are
102 vectors of *Bartonella* in bats. We hypothesize that limitation of parasite dispersal will result in
103 stochastic losses of rare *Bartonella* species and increases in community beta diversity due to
104 ecological drift, and shifts in the rank abundance of *Bartonella* communities due to local
105 selection. Finally, potential interactions among *Bartonella* species will be detectable based on
106 coinfection frequencies, specifically evidence of competition and/or facilitation. This work
107 expands our understanding of *Bartonella* dynamics in natural communities, particularly in bats
108 and their ectoparasites. More broadly, this experiment deepens our understanding of the

109 processes that affect parasite communities, patterns which may be compared with those seen in
110 communities of free-living or mutualistic organisms.

111

112 **Materials and methods**

113 *Study system*

114 *Eidolon helvum* (Chiroptera: Pteropodidae) is a long-lived, tree-roosting bat species that
115 can form enormous colonies during the local dry season (Fahr et al., 2015; Hayman et al., 2012).

116 Bat flies (*Cyclopodia greefi*; Diptera: Nycteribiidae) are obligate blood-feeding ectoparasites of
117 bats. The flies are wingless but can move among hosts within densely populated roosts.

118 *Bartonella* spp. (Alphaproteobacteria: Rhizobiales) are intracellular bacteria that infect mammals
119 and are transmitted by blood-feeding arthropods (Harms & Dehio, 2012). At least six distinct

120 *Bartonella* species have been previously described in *E. helvum* (Bai et al., 2015; Kosoy et al.,
121 2010) and the same species plus additional variants have been detected in *C. greefi* (Billeter et al.,
122 2012; Kamani et al., 2014). Based on these data and other studies (Brook et al., 2015; Morse et
123 al., 2012; Moskaluk et al., 2018), it has been proposed that bat flies are vectors of *Bartonella* spp.
124 in bats, but no experimental studies have been performed to demonstrate their competence.

125 Materials for this study come from a captive population of *E. helvum* bats in Accra,
126 Ghana (Baker et al., 2014). Briefly, the captive facility is a double-fenced hexagonal 27.5 m
127 diameter and 3.5 m high structure; a solid metal roof and cladding at the base prevent contact
128 with other animals. The captive population was founded by three cohorts (Table S1) of mixed age
129 and sex (n = 78) collected from a large seasonal colony in Accra (Hayman et al., 2012). The
130 cohorts entered the colony in July 2009, November 2009, and January 2010; two additional
131 cohorts were born in captivity in April 2010 (produced by mating between wild bats before

132 entering the colony) and 2011 (produced by mating in captivity). All 13 captive-born neonates
133 were matched to the dam they were attached to at the first sampling point after birth. Ethics
134 approval for bat capture and the fly reintroduction experiment was obtained from the Zoological
135 Society of London Ethics Committee (WLE/0467), the Veterinary Services Directorate of Ghana,
136 and the Wildlife Division of the Forestry Commission of Ghana.

137 Bats were assigned to age classes and sex upon entry to the colony and afterward
138 according to approximate birth date and secondary sexual characteristics (Peel et al., 2016):
139 neonate, juvenile, sexually immature adult, and sexually mature adult. Passive integrated
140 transponder (PIT) tags were implanted in each bat either at entry or shortly after birth to uniquely
141 identify each bat and adult bats additionally received necklaces with alphanumeric codes.
142 Although 112 total bats entered the colony, 25 bats left the colony either through recorded
143 mortality (n = 12) or presumed mortality after being recorded missing for ≥ 3 sampling points (n =
144 13). Furthermore, not all bats had complete sample histories throughout the experiment because
145 they intermittently escaped capture for processing.

146 Blood samples were taken from the captive bats every two months in 2009 and 2010 and
147 every four months in 2011 (Table S1; see Appendix 1 for sampling protocol). On 6 March 2010
148 (denoted M10, day 221), a sample of bat flies (*C. greffi*; n = 28) was removed from the colony
149 for testing for the presence of *Bartonella* DNA and from that point forward the fly population
150 was observed to decline. Subsequent to this, it is assumed that little among-host bacterial
151 transmission was occurring. To test the effect of restoring transmission on *Bartonella* community
152 dynamics and to provide evidence that bat flies are vectors, bat flies were experimentally
153 reintroduced to the colony. On 17 January 2012 (J12, day 903), a sample of adult bat flies and
154 nymphs was taken from the original wild source colony of bats (n = 51), along with matched

155 blood samples from donor bats ($n = 42$), and the flies were randomly assigned to approximately
156 half the bats in the colony ($n = 40$; 1–4 flies per bat) while additional bat flies were collected for
157 testing for the presence of *Bartonella* DNA ($n = 18$). Blood samples from captive bats were
158 subsequently taken at three additional time points after the reintroduction of flies: 31 January
159 2012, 14 February 2012, and 15 March 2012. In total, 910 blood samples were taken from the
160 captive colony over 14 time points from 2009 to 2012 (a period of 961 days), of which 905
161 samples could be definitively assigned to an individual by PIT tag or necklace ID. An additional
162 50 blood samples and 18 flies were taken from wild bats on J12.

163

164 *Bacterial detection and gene sequencing*

165 The focus of this study was on changes in *Bartonella* infection prevalence and the relative
166 abundance of different *Bartonella* species in bats, so a molecular detection and sequencing
167 approach capable of distinguishing coinfecting species was used. Bat blood and fly samples were
168 tested for the presence of *Bartonella* DNA using a multi-locus PCR platform (Bai et al., 2016)
169 targeting fragments of the 16S–23S ribosomal RNA intergenic spacer region (ITS), citrate
170 synthase gene (*gltA*), and cell division protein gene (*ftsZ*). Each of these loci is capable of
171 distinguishing among *Bartonella* species and subspecies (La Scola et al., 2003), but may have
172 amplification biases toward different *Bartonella* species in a sample (Himsworth et al., 2020;
173 Kosoy et al., 2018). Thus, the purpose of this multi-locus approach was to confirm the detection
174 of *Bartonella* DNA and to indicate across loci whether infections with multiple species were
175 present. Further quantification of *Bartonella* infection load was performed using real-time PCR
176 targeting the transfer-messenger RNA (*ssrA*). Sequences were verified as *Bartonella* spp. using
177 the Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

178 Samples were only considered positive if a significant match was observed, even if there was a
179 positive real-time PCR result (cycle threshold value [Ct] < 40). *Bartonella* sequences with
180 multiple peaks in the electropherogram were separated into two or more distinct sequences by
181 comparison with previously obtained *Bartonella* sequences from *E. helvum* and *C. greefi* (Bai et
182 al., 2015; Billeter et al., 2012). Due to the frequency of multiple sequences obtained from these
183 loci, conflicting sequences across genes were interpreted as evidence of coinfection rather than
184 homologous recombination, and thus we report counts of sequences from distinct *Bartonella*
185 species within a sample as recommended by Kosoy et al. (2018). All variants of *Bartonella*
186 sequences sharing <95% sequence similarity with previously identified *Bartonella* species were
187 submitted to GenBank. Additional details on bacterial detection and phylogenetic analysis are
188 provided in Appendix 1.

189

190 *Data recording and statistical analyses*

191 Relevant measures of *Bartonella* infection prevalence, infection load, and diversity were
192 recorded or calculated to assess changes that occurred during the experiment, including before
193 and after the reintroduction of bat flies to the captive colony. *Bartonella* infection prevalence
194 within the captive bat colony, in sampled wild and captive flies, and from wild bats was reported
195 based on the number of tested bats or flies that were positive at one or more loci (ITS, *gltA*, *ftsZ*,
196 *ssrA*). Wilson scores were used to calculate 95% confidence intervals for single infection and
197 coinfection prevalence. *Bartonella* alpha diversity was measured by *Bartonella* species richness
198 and Shannon number, i.e., the effective number of species or the exponent of Shannon's diversity
199 index (Jost, 2006); species richness within each sample based on the number of loci positive was
200 also recorded. *Bartonella* species relative abundances were calculated from the total number of

201 sequences obtained across all loci, including separate sequences obtained from the same locus. A
202 custom bootstrapping procedure with 1000 samples from the observed multinomial distribution
203 of *Bartonella* species relative abundances was used to estimate 95% confidence intervals around
204 measures of alpha diversity. *Bartonella* beta diversity was measured across sampled bats and flies
205 using the binomial index option of the `vegdist` function in the R package *vegan* (Oksanen et al.,
206 2019; R Core Team, 2020). Infection load was recorded as the number of loci positive and real-
207 time PCR Ct value for each sample. Additionally, for each bat the time until becoming infected
208 after first entering the colony and the duration of infection for the most persistent *Bartonella*
209 species were recorded. These measures help to track whether certain demographic groups are
210 more affected by the reintroduction of flies and to compare with changes in relative abundances
211 of *Bartonella* species over time, respectively. Change points in *Bartonella* prevalence, infection
212 load, and diversity measures were detected with segmented regression using the R *segmented*
213 package (Muggeo, 2020). Chi-square or Fisher's exact tests were performed to compare changes
214 in infection status for bats that did or did not receive bat flies on J12. Multinomial and binomial
215 likelihood ratio (LR) tests adapted from Pepin et al. (2013) were performed to find statistical
216 associations between coinfecting *Bartonella* species and to detect changes in the relative
217 abundances of *Bartonella* species during the study period. For additional details regarding
218 regression analyses and likelihood ratio tests, see Appendix 1.

219

220 **Results**

221 *Phylogenetic analysis of detected bacteria*

222 *Bartonella* infections in bats and bat flies were identified as six previously characterized
223 species based on ITS, *gltA*, and *ftsZ* sequences: *Bartonella* spp. E1–E5 and Ew (Bai et al., 2015;

224 Kosoy et al., 2010). Two additional genogroups identified by *gltA* sequences, *Bartonella* spp.
225 Eh6 and Eh7 (Figure S1), were similar to sequences previously obtained from *C. greefi* collected
226 from *E. helvum* in Ghana and two islands in the Gulf of Guinea (Billeter et al., 2012).
227 Phylogenetic analysis of concatenated *ftsZ* and *gltA* sequences distinguished Eh6 and Eh7 from
228 other *Bartonella* species associated with *E. helvum* or other bat species (Figure S4). See
229 Appendix 2 for more details on phylogenetic analysis.

230

231 *Bartonella* infection prevalence and effects of bat fly reintroduction

232 As predicted, *Bartonella* prevalence in the captive colony changed with the population
233 density of bat flies. *Bartonella* prevalence in the first three cohorts was high at colony entry, then
234 declined concurrently with the observed decline in the bat fly population (Figure 1A). After flies
235 were reintroduced, prevalence increased from 31% on day 903 to 48% on day 961. This change is
236 reflected in the segmented regression analysis (Figure S6A; Table S4) with a shift from positive
237 to negative slope near M10 (day 221) and a shift from negative to positive slope near J12 (day
238 903). The trend in *Bartonella* prevalence in the colony over time was similar if bats were
239 considered positive for *Bartonella* with a threshold of at least one, at least two, at least three, or
240 all genetic markers being positive (Figure S7).

241 The effect of bat fly reintroduction affected some age classes of bats more than others.
242 Most sexually immature and sexually mature adult bats initially entered the colony infected
243 (Figure S8A). All sexually immature bats were infected at entry and at the end of the study, but
244 there was an increase in the proportion of sexually mature adult bats ($\chi^2 = 3.2$, $df = 1$, $P = 0.038$)
245 infected by the end of the study compared to the start. Bats born into the colony in 2010 and 2011

246 were *Bartonella*-negative at first sampling. By the end of the experiment, 88% of these bats had
247 become infected (Figure S8A), a very significant increase ($\chi^2 = 48.2$, $df = 1$, $P < 0.001$).

248 Out of the 53 bats that were negative on J12, 32 bats (60.4%) became positive after flies
249 were reintroduced ($\chi^2 = 43$, $df = 1$, $P < 0.001$). The effect of flies on prevalence was much more
250 pronounced for bats that were born into the colony in 2010 and 2011 than for adult bats: 16/17
251 (94.1%) captive-born cohort bats became positive after reintroduction versus 16/36 (44.4%) wild-
252 caught cohort bats ($\chi^2 = 9.9$, $df = 1$, $P < 0.001$). Including bats that were already positive on J12,
253 48/84 (57.1%) either became positive or changed *Bartonella* species after fly reintroduction ($\chi^2 =$
254 64.4, $df = 1$, $P < 0.001$). This effect was greater for captive-born cohort bats than for wild-caught
255 cohort bats: 22/28 (78.6%) late cohort bats versus 26/30 (46.4%) early cohort bats ($\chi^2 = 6.6$, $df =$
256 1, $P = 0.005$). However, when comparing bats that received flies versus those that did not (i.e.,
257 cases versus controls), there were no significant differences between groups in their change in
258 infection status after fly reintroduction (see Appendix 2 for details). Thus, the effect of bat fly
259 reintroduction was only observable at the population-level infection prevalence and within age
260 classes, but not for individual bats.

261 Bat fly reintroduction had similar effects on measures of infection load in the colony.
262 Infection load in each sample as measured by RT-PCR cycle threshold (Ct) values (Figure 1B)
263 and the number of positive genetic markers per sample (Figure S9A) reached a peak on M10,
264 then declined before sharply increasing after the reintroduction of flies. This trend is reflected in
265 the segmented regression of both measures, with a shift from positive to negative slope near day
266 221 and a shift from negative to positive slope near day 903 (Figure S6B,C; Table S4).
267 Coinfection prevalence also showed a peak near M10 and declined until July 2011 (day 715)
268 when it began to increase again (Figure S9B). However, neither of the shifts in slope for

269 coinfection prevalence were statistically significant (Figure S6D; Table S4). For details on
270 prevalence and load in bat flies and wild bats collected on M10 and J12, see Appendix 2.

271

272 *Patterns of Bartonella diversity*

273 Similar to infection prevalence and load, *Bartonella* diversity measures changed in
274 response to bat fly population density. *Bartonella* diversity was measured at two scales, at the
275 colony level and at the individual host level. *Bartonella* species richness and evenness (Shannon
276 index) measured colony-level alpha diversity. The number of *Bartonella* species in an individual
277 sample and beta diversity (binomial index) measured individual-level diversity. Diversity
278 measures showed qualitatively similar patterns during the early phase of the experiment (Figure
279 2A; Figure S10): an initial increase with the entry of the first three cohorts into the colony
280 reaching a maximum in January 2010 followed by a decline. Diversity measures increased again
281 until the reintroduction of flies on J12 and then declined slightly (or remained flat in the case of
282 species richness). The observed trends were only partially reflected by segmented regression
283 breakpoints. Segmented regression detected only one breakpoint each in the timelines for species
284 richness, species evenness, and the number of *Bartonella* species in an individual sample (Table
285 S4). A shift from positive to negative slope was detected in January 2010 for species richness
286 (Figure S11A) whereas a change from negative to positive slope was detected for species
287 evenness and the number of species in an individual sample between November 2010 and March
288 2011 (Figure S11B,C; Figure S12A). There were two significant breakpoints detected in the
289 timeline of beta diversity, changing from negative to positive slope in July 2010 and from
290 positive to negative slope in January 2012 (Figure S12B; Table S4). For details on diversity
291 measures in bat flies and wild bats collected on M10 and J12, see Appendix 2.

292

293 *Shift in Bartonella species abundance*

294 *Bartonella* species observed in the colony varied in their relative abundance, with an
295 apparent shift in the dominant species during the study (Figure 2B). While rarer species E1, E2,
296 and Eh7 were not observed at all time points, E1 and E2 were consistently observed over the
297 duration of the study. In contrast, the rarest species Eh7 was not observed after July 2010, even
298 after flies were reintroduced to the colony. Species Eh6 was also uncommonly observed during
299 the study, went unobserved for three time points in 2012, but was observed again in March 2012.

300 As noted above, beta diversity decreased after January 2010 when the bat fly population
301 was decreasing, reached another maximum in January 2012, and then decreased again after flies
302 were reintroduced (Figure 2A). These decreases in beta diversity correspond with periods of
303 expansion by some species within the colony that appear to homogenize beta diversity. During a
304 period from January 2010 to July 2011, Ew became the most abundant species in the colony
305 (Figure 2B). Another measure of this species' dominance in the colony is the duration of its
306 infections in individual bats. For each individual bat that was sampled more than once and was
307 recorded as having the same *Bartonella* species for a sequential period, we tabulated which
308 species was present for the most time points (Figure 3). Among *Bartonella* species, Ew was the
309 longest lasting infection in the highest number of bats ($n = 40$). The infection durations for this
310 species ranged from 37 to 610 days with a median of 145 days.

311 Beginning around March 2011, the relative abundance of Ew began declining while
312 species E1, E2, and E5 increased (Figure 2B). Dividing the study into two parts – before flies
313 were introduced (July 2009 to July 2011) and after flies were introduced (J12 and after) – a clear
314 difference in the rank abundance of *Bartonella* species was observed (Figure 2C). This shift in

315 abundance after the introduction of flies was significant according to a multinomial LR test ($D =$
316 350.1 , $df = 7$, $P < 0.001$) and individual binomial LR tests for all species (Table S5). Significant
317 differences were also observed in the relative abundances between bat flies and sampled bat
318 populations on M10 and J12 (Figure 2D,E; Table S6). Patterns in the occurrence of species over
319 time and relevant tests of differences in the *Bartonella* community were similar if the relative
320 counts (presence/absence of species across any marker rather than counts across markers) were
321 used instead of relative abundance (Figure S13). For details on this and tests of differences in the
322 relative abundance of species in bat flies and wild bats, see Appendix 2.

323

324 *Interactions between Bartonella species*

325 Using multinomial and binomial LR tests on coinfection frequencies, there was evidence
326 of both negative and positive interactions between *Bartonella* species over the period of the
327 experiment (Figure 4). Bats infected with Ew were significantly less likely to be coinfecting with
328 E2, E3, and E5; a reciprocal negative effect on Ew from these species was not detected. Related
329 to this, the proportion of Ew infections that were also coinfections was low (30%) considering its
330 high relative abundance in the population over time (Figure 2B). Species E1 and Eh6 had a
331 reciprocal negative effect on each other. Reciprocal positive effects (i.e., more coinfections than
332 expected) were found between species E3 and E5 and species E1 and E5. Also, bats were more
333 likely to be coinfecting with Ew if they were already infected with E1, but there was no significant
334 reciprocal effect of Ew on E1 (Figure 4).

335

336 **Discussion**

337 Parasites do not infect hosts in isolation but instead form diverse communities in hosts
338 that vary over time. However, it is unclear if the same forces that affect diversity in communities
339 of free-living organisms act in the same way or with different strengths in parasite communities.
340 This study tested how well predictions of community ecology theory apply to host-vector-
341 parasite systems through a unique approach that manipulated parasite dispersal among hosts
342 within the population by changing the population density of the putative vector. Restriction of
343 parasite dispersal minimized the effect of among-host transmission on *Bartonella* communities
344 within individual hosts, thereby allowing the effects of ecological drift and selection on parasite
345 community diversity to be measured. At the same time, observed trends in the prevalence and
346 diversity of *Bartonella* infections within the colony over the course of vector population decline
347 and reintroduction indicate that bat flies are biological vectors of *Bartonella* in bats. Overall, the
348 experiment shows that *Bartonella* communities are affected by dispersal, drift, and selection in
349 similar ways to free-living organisms, although numerous forms of ecological selection might be
350 acting simultaneously.

351 We first hypothesized that *Bartonella* communities in the colony would respond to
352 changes in among-host dispersal/transmission by bat flies. Specifically, we predicted that
353 infection prevalence and diversity would decline concurrently with the bat fly population and
354 then increase upon reintroduction of flies. The results indicate that *Bartonella* prevalence and
355 infection load declined along with the bat fly population, then increased when flies were
356 reintroduced in January 2012 (Figure 1). This effect was seen across the whole population but
357 had a stronger effect on young bats born in the colony, likely attributable to their lack of prior
358 exposure to *Bartonella* while flies were in low density. Only a few vectors of *Bartonella* bacteria

359 have been confirmed through controlled exposure of hosts to infected vectors (Morick et al.,
360 2013; Tsai et al., 2011). A previous study by Jardine et al. (2006) demonstrated declines in
361 *Bartonella* prevalence after an experimental insecticide treatment reduced flea densities on
362 Richardson's ground squirrels (*Spermophilus richardsonii*), indicating that fleas are important
363 vectors of *Bartonella*. Similar to this study, our results confirm that bat flies are likely vectors of
364 *Bartonella* bacteria in bats.

365 *Bartonella* diversity also decreased over the corresponding period when flies were
366 declining (Figure 2A; Figure S10). This decline may be attributed to the stochastic loss of rare
367 species and the increase in abundance of some species, specifically Ew, through persistent
368 infection (Figure 2B). Interestingly, all diversity measures actually increased prior to the
369 reintroduction of flies, reaching a local peak in diversity in January 2012 before declining. This
370 second decline could be attributed to the decline of the dominant Ew, allowing potentially latent
371 infections by other species (E1, E2, E3, E5) to emerge as the dominant species infecting the bat
372 population. The dominance of these species continued after flies were reintroduced and among-
373 host transmission was restored, thus causing a short decline in diversity measures. These patterns
374 indicate that dispersal of infections by flies is key to the long-term maintenance of *Bartonella*
375 community diversity in bats.

376 While the experiment was originally designed to split bats into treatment versus control
377 groups to assess the effect of bat fly reintroduction on changes in *Bartonella* infection status, this
378 was not successful. Bats that received flies were not more likely to become infected or change
379 *Bartonella* species after reintroduction. This probably occurred because bat flies did not remain
380 on the bat they were placed on and instead moved among individuals in the colony. This would
381 produce the poor correlation between infection status of bats and flies, as seen in the results

382 presented and those of Becker et al. in vampire bats (2018). Nevertheless, this study establishes
383 that the loss and reintroduction of bat fly vectors is associated with changes in *Bartonella*
384 infection and diversity at the host population level.

385 We also hypothesized that limitation of parasite dispersal would result in stochastic losses
386 of rare *Bartonella* species and changes in community beta diversity via ecological drift and shifts
387 in the rank abundance of *Bartonella* communities due to local selection. The rarest species in the
388 community, *Bartonella* species Eh7, was lost during the course of the study and was not restored
389 when flies were reintroduced. This failure was likely due to a sampling effect, wherein flies carry
390 only a subset of *Bartonella* species (Figure 2D,E), therefore limiting opportunities for effective
391 dispersal of rare species. As noted above, beta diversity did not exhibit the expected increase
392 when the fly population declined. Instead there was a decrease in beta diversity due to the
393 dominance of species Ew (Figure 2A). This dominance of Ew was the most conspicuous trend in
394 the dynamics of the *Bartonella* community over most of the study, except for the end of the
395 experiment when there was a shift towards the next most abundant species, E5, and other lower
396 ranked species (Figure 2B). This shift towards E5 and the decline in Ew occurred before the
397 reintroduction of flies and was independent of the effects of among-host dispersal (due to the low
398 density of flies at this time). We speculate that this is an emergent pattern due to within-host
399 selection against Ew by the host immune system. Specifically, as Ew came to dominate within
400 the population and in individual bats, it may have become the primary target of host immune
401 responses. As Ew was eliminated, this allowed for the emergence of other latent infections within
402 coinfecting bats. Thus, without dispersal of *Bartonella* species by bat fly vectors, we speculate
403 that ecological drift and selection by the host immune system may cause observable changes in
404 bacterial communities.

405 Finally, we expected that potential interactions among *Bartonella* species would be
406 detectable based on coinfection frequencies, providing evidence of competition or facilitation in
407 pathogen communities. While most interactions were not significant, species Ew has negative
408 effects on several species and typically has few coinfections (Figure 4). In contrast, positive
409 effects were observed between species E1, E3, and E5, which show a much higher frequency of
410 coinfection. These results indicate that parasitic bacteria like *Bartonella* do have measurable
411 ecological interactions which are not uniformly competitive. These positive interactions could
412 have played a role in the replacement of Ew with E5 and other species late in the study.

413 From just a single experiment, we can make several inferences about the ecology of
414 *Bartonella* infections in bats. First, they can be persistent, lasting potentially hundreds of days.
415 Other studies have alluded to the possibility of persistent *Bartonella* infection with periodic
416 recrudescence in rodents (Bai et al., 2011; Goodrich et al., 2020; Kosoy et al., 2004) and bats
417 (Becker et al., 2018); however, these studies were conducted in open populations where
418 reinfection by vectors was likely frequent. Although we cannot rule out that some reinfection
419 occurred due to the remnant bat fly population in the colony, the decline in bat fly density should
420 have reduced reinfections relative to studies of wild populations. Second, *Bartonella* community
421 diversity can be driven by dispersal, drift, and selection. The current study has shown that when
422 dispersal is limited, the effects of ecological drift and selection can be more apparent. Two types
423 of ecological selection can occur in these parasite populations, either through interactions with
424 the host immune system or through interspecific interactions. As noted above, the immune
425 system may lead to periodic selection against the dominant infection, a negative frequency-
426 dependent mechanism that might help maintain diverse parasite communities (Fallon et al.,
427 2004).

428 Dominance appears to be a similar facet of the composition of bacterial communities as it
429 is in free-living organisms (Smith & Knapp, 2003). The dominance of Ew may thus stem from
430 multiple facets of its ecology. First, it appears to be persistent within bats (Figure 3) and second,
431 it appears to be readily taken up by flies (Figure 2D,E). We note that Ew is also the most clonal,
432 i.e., genetically homogenous, species in the community and might be a more recently evolved or
433 introduced species in *E. helvum* (Bai et al., 2015). While there was no evidence that Ew caused
434 higher infection loads (by Ct value or number of markers positive), the resolution of our sampling
435 protocol probably was not high enough to detect this. Future studies should inspect growth curves
436 throughout the infection cycle to see if Ew has any growth advantage. Other forms of interference
437 or resource competition must be explored further, perhaps through controlled infection
438 experiments.

439 Future work within this system might involve controlled exposure of *Bartonella*-negative
440 bats and confirmation of the exposure route. Alternative routes might include bat fly bite,
441 requiring tropism of the bacteria to the salivary glands, or contamination through bat fly feces,
442 requiring replication in the fly gut and persistent shedding of viable bacteria in feces. Additional
443 studies could examine immune function in bats (Boughton et al., 2011) in response to *Bartonella*
444 infection to confirm the existence of frequency-dependent selection against *Bartonella* species
445 and to help determine the appropriate epidemiological models to explain *Bartonella* infection
446 dynamics (Brook et al., 2017).

447 This study has contributed to a more comprehensive understanding of the ecology of
448 *Bartonella* species in bats and connects with broader community ecology theory developed in
449 free-living and symbiotic organisms (Costello et al., 2012; Miller et al., 2018; Vellend, 2010).
450 Limitation of dispersal in this experiment led to declines in local species diversity in individual

451 bats, a pattern that fits well with predictions from patch dynamics or mass effects models of
452 metacommunities (Leibold et al., 2004). The results also show that not all bacterial interactions
453 are negative, even those that presumably share the same niche. This parallels the recognized
454 importance of positive species interactions in plant communities (Bertness & Callaway, 1994)
455 and among bacterial taxa in animal microbiomes and aquatic habitats (Faust et al., 2012; Hegde
456 et al., 2018; Ju & Zhang, 2015). A recent study by Gutiérrez et al. (2018) on *Bartonella*
457 infections in desert rodents showed a mixture of negative, neutral, and positive interactions
458 similar to the present study. Theoretical and experimental studies suggest that communities
459 remain stable through a predominance of neutral or weak species interactions that can attenuate
460 large competitive or facultative effects (Aschehoug & Callaway, 2015; McCann, 2000). Weak
461 interactions, paired with the frequency-dependent selection discussed above, could provide a
462 model for understanding how *Bartonella* species and other parasitic microorganisms coexist in
463 communities within their hosts. Such mechanisms could allow bacteria to share a niche or split it
464 temporally, which could lead to periodic shifts in the dominant species but maintain the
465 community as a whole. Future work using this system and similar longitudinal studies on other
466 pathogens in natural host populations could lead to additional insights on the nature of
467 microorganismal communities and the broad ecological processes that act across taxonomic and
468 spatial scales.

469

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478

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644 **Data accessibility**

645 The data that supports the findings of this study are available in the supplementary
646 material of this article. Representative sequences for *Bartonella* genogroups Eh6 and Eh7 and
647 two *Rickettsia* sequences from *E. helvum* and *C. greefti* have been submitted to GenBank under
648 the accession numbers MN249715–MN249720, MN250730–MN250788, and MN255799–

649 MN255800. Phylogenetic trees, R code, and additional data sheets will be made available on
650 Dryad.

651

652 **Author contributions**

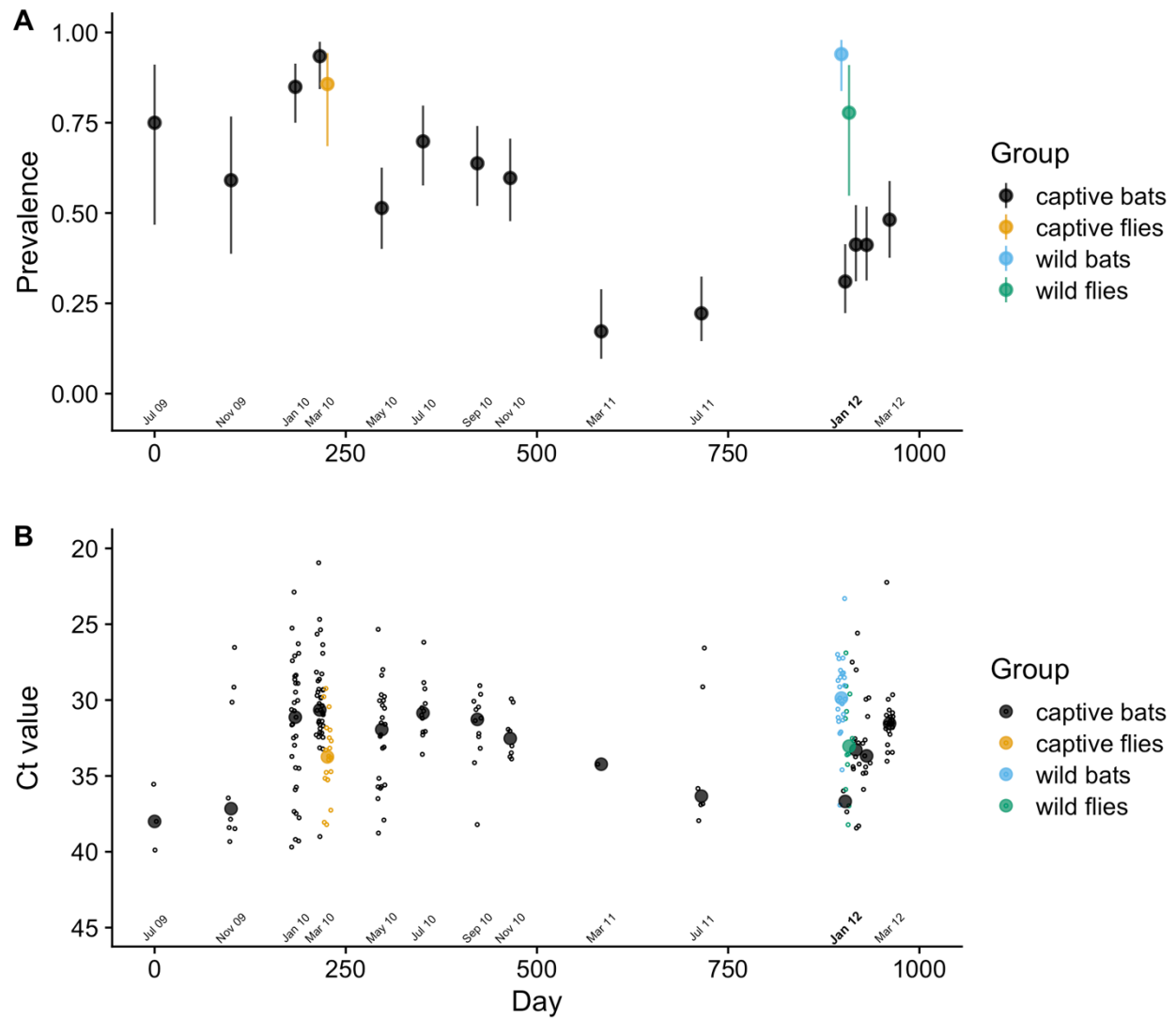
653 DTSH, JLNW, AAC, YN, and RS designed research; CDM, MYK, YB, LMO, RS, and
654 DTSH performed research; CDM analyzed data; CDM, CTW, and DTSH wrote the paper; all
655 authors contributed to and approved the final version of the paper.

656

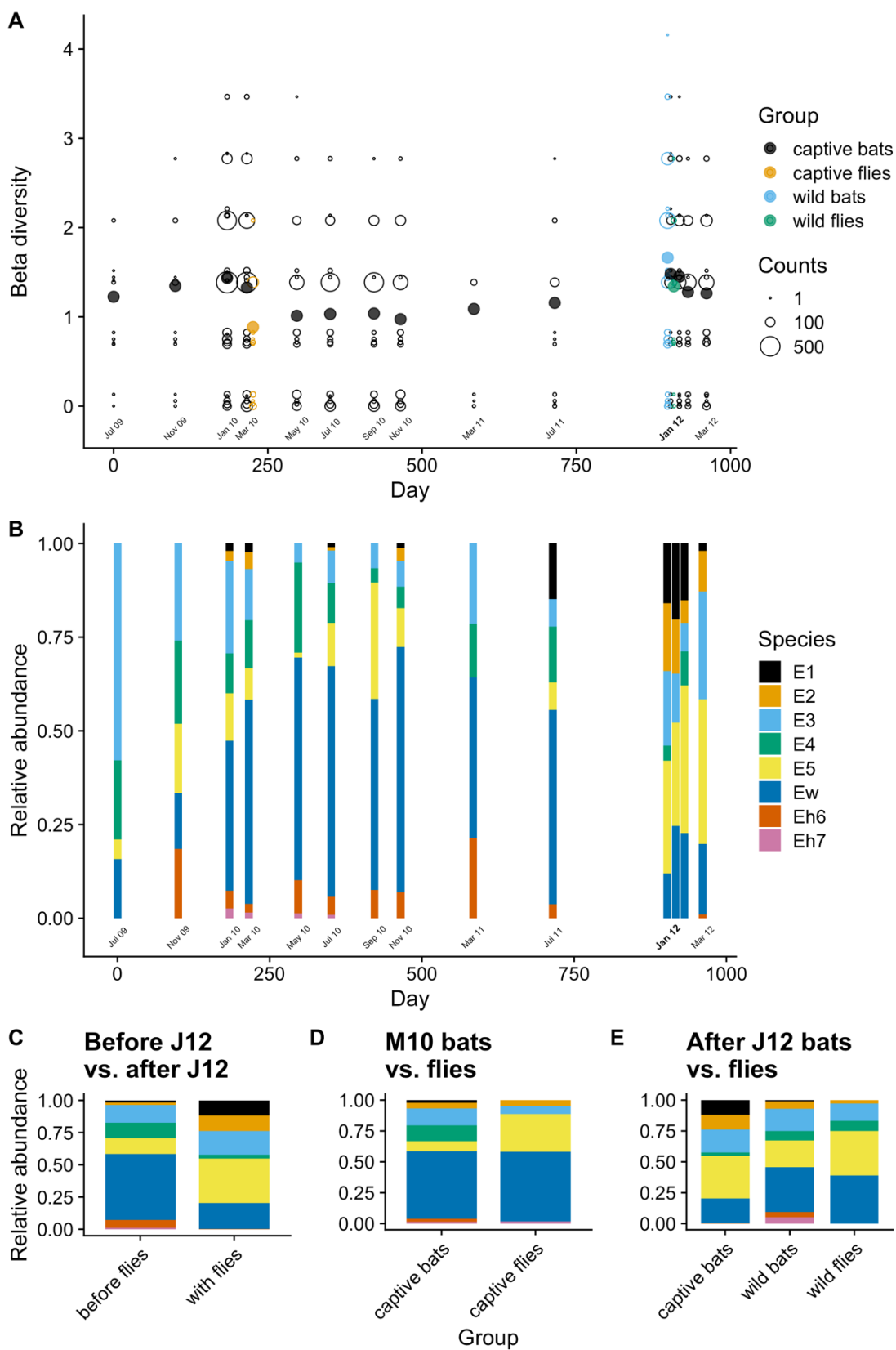
657 **Conflict of interest**

658 The authors declare no conflicts of interest.

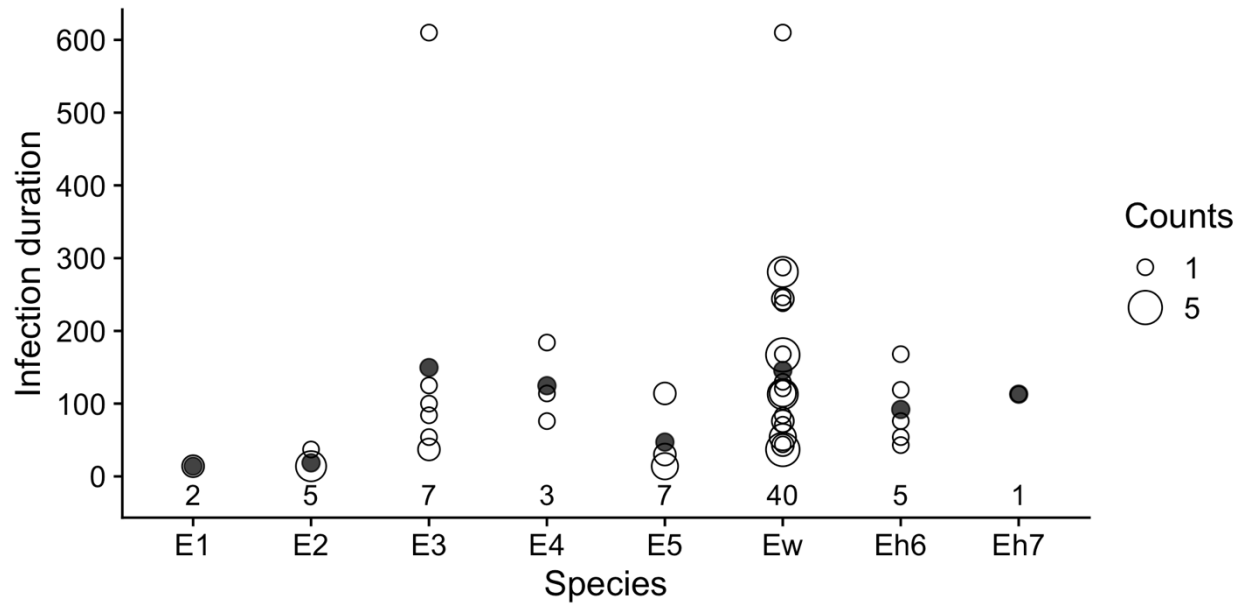
659



660
661 **Figure 1.** *Bartonella* infection prevalence and load in a captive colony of *E. helvum* over time.
662 (A) Bats and bat flies were considered positive if a *Bartonella* sequence was obtained from one
663 or more genetic markers. Wilson score 95% confidence intervals were drawn around prevalence
664 estimates at each sampling time point. (B) Only points with RT-PCR Ct values < 40 are shown.
665 Mean Ct values calculated at each time point are drawn as a filled circles over the data (open
666 circles). The month labeled in bold font on the x-axis shows when bat flies were reintroduced.
667



669 **Figure 2.** Changes in *Bartonella* beta diversity and the relative abundance of *Bartonella* species
670 in bats and bat flies over time. Beta diversity (A) was calculated using the binomial index
671 comparing across all infected bats and bat flies in the colony. Data for individuals are shown as
672 open circles for each individual with the width proportional to the number of individuals with the
673 same index value. Solid circles show the mean values. Relative abundance (B) at each time point
674 was estimated from the total number of counts for each *Bartonella* species based on sequences
675 from ITS, *gltA*, and *ftsZ*. For panels A and B, the month labeled in bold font on the x-axis shows
676 when bat flies were reintroduced. Tests for differences in the relative abundance of species were
677 performed between bats in the captive colony before and after bat flies were reintroduced on 17
678 January 2012 (C); between bat flies sampled from the colony and the captive bat population in
679 March 2010 (D); and between bat flies and wild bats sampled on 17 January 2012 and the captive
680 colony population after flies were reintroduced (E).
681



682
683 **Figure 3.** Duration of *Bartonella* sp. infections in serially infected individuals. For each
684 *Bartonella* species, the numbers below the points are counts of individual bats that had the
685 *Bartonella* species as its longest lasting infection (i.e., the *Bartonella* species was present for the
686 most sequential time points). The infection durations in days for all serially infected bats are
687 plotted as open circles with the width proportional to the number of individuals with the same
688 infection duration. Solid circles indicate the mean duration.
689

	E1	E2	E3	E4	E5	Ew	Eh6	Eh7	Multinomial P	Proportion coinfections
E1	12	3	7	2	11	8	0	0	0.11	0.72
E2	3	16	8	2	5	4	2	0	0.38	0.60
E3	7	8	46	9	23	20	6	0	0.084	0.61
E4	2	2	9	30	3	17	1	1	0.51	0.54
E5	11	5	23	3	55	14	2	0	0.0014	0.51
Ew	8	4	20	17	14	165	7	1	0.054	0.3
Eh6	0	2	6	1	2	7	27	0	0.15	0.4
Eh7	0	0	0	1	0	1	0	5	0.8	0.29

More than expected
 Less than expected

690
 691 **Figure 4.** Patterns of *Bartonella* species coinfection. Rows are the focal species and columns are
 692 the partner infections. Numbers in the boxes are counts of coinfections between each pair of
 693 species; single infection counts for each species are on the diagonal. Black boxes show
 694 coinfections that occurred more frequently than expected, grey boxes show those that occurred
 695 less frequently than expected, and white boxes showed no significant pattern. Expected counts
 696 were based on the frequency of single and double infections of each *Bartonella* species, and
 697 significance was based on multinomial and binomial tests. The proportion of infections by each
 698 *Bartonella* species that were also coinfections are shown in the last column.