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The role of behavioural heterogeneity on infection patterns: implications for pathogen transmission

Laurie Dizney

University of Portland, dizney@up.edu

M. Denise Dearing

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1 Animals infected with pathogens often differ in behaviour from their uninfected
2 counterparts, and these differences may be key to understanding zoonotic pathogen
3 transmission. To explore behavioural heterogeneity and its role in pathogen transmission,
4 we studied deer mice (*Peromyscus maniculatus*) under field conditions. Deer mice are the
5 natural host of *Sin Nombre* virus (SNV), a zoonotic pathogen with high human mortality.
6 We live-trapped mice in May, July and September of 2009 and 2010, marked captures
7 with passive integrated transponder (PIT) tags, recorded physical characteristics and
8 collected blood samples for SNV analysis. For four nights after each trapping session, we
9 observed behaviour with a novel surveillance system of nine camera stations, each
10 consisting of a foraging tray, infrared camera, PIT antenna and data logger. We found
11 that deer mice infected with SNV (30.0%) engaged more frequently in behaviours that
12 increased the probability of intraspecific encounters and SNV transmission than
13 uninfected. When deer mice were categorized as bold (31.7%) or shy (68.3%) based on
14 these behaviours, bold behaviour was predictive of positive SNV status. Bold deer mice
15 were three times more likely to be infected with SNV than shy deer mice. These results
16 suggest that a small percentage of bold individuals are responsible for a majority of SNV
17 transmission events, and that behavioural phenotype is an important consideration in
18 transmission dynamics of zoonotic diseases.

19

20 *Keywords:* aggressive interactions, disease ecology, disease transmission, hantavirus,
21 risky behaviour, zoonotic disease

22

23 Emerging infectious diseases (EIDs) have been increasing in the last 30 years (Jones
24 et al. 2008), threatening the health of humans and wildlife alike (Daszak et al. 2000). It is
25 estimated that 75% of EIDs are zoonotic (Taylor et al. 2001), meaning they originate in
26 wildlife. To determine which factors increase prevalence in host populations, and thus
27 increase human risk, it is essential to understand how zoonotic pathogens are spread. Yet,
28 transmission dynamics are largely unknown for most wildlife species. While host
29 susceptibility is likely important (Hawley and Altizer 2011), host behaviour is an intrinsic
30 part of transmission dynamics, particularly for directly transmitted pathogens. Behaviour
31 of animals infected with pathogens often differs from the population at large, sometimes
32 prior to infection, but other times as the result of infection (Lafferty and Morris 1996;
33 Berdoy et al. 2000; Klein 2003; Luong et al. 2011). Such differences in behaviour are
34 important, as it typically results in a subset of the population being responsible for the
35 majority of transmission, as has been documented in the human pathogens SARS and
36 HIV (May and Anderson 1987; Dye and Gay 2003; Lloyd-Smith et al. 2005).
37 Heterogeneity in behavioural patterns has been examined far less frequently in wildlife
38 (Perkins et al. 2003; Kilpatrick et al. 2006; Clay et al. 2009) yet it may be key to
39 understanding transmission.

40

41 We studied the behaviour of a rodent with respect to hantavirus infection status to
42 investigate the behaviour underlying transmission dynamics of zoonoses within host
43 populations. Hantaviruses are emerging infectious diseases with a worldwide distribution,
44 causing hundreds of thousands of hospitalizations and hundreds of deaths annually (Bi
45 and Roth 2008; Heyman et al. 2009) The hantavirus of greatest public health concern in

46 North America is *Sin Nombre* virus (SNV), which can cause Hantavirus Pulmonary
47 Syndrome (HPS) in humans. Since its discovery in 1993, 617 cases of HPS have been
48 confirmed in the United States, with a 35 % mortality rate
49 (<http://www.cdc.gov/hantavirus/>).

50

51 Deer mice (*Peromyscus maniculatus*) are the hosts of SNV (Childs et al. 1994; Nichol
52 et al. 1993), and are widely distributed throughout North America (Hall 1981). Deer mice
53 have overlapping home ranges. Males show increased aggression during the breeding
54 season, as do females when defending their young (Wolff 1989). SNV infection in deer
55 mice is chronic and appears to be asymptomatic (Botten et al. 2003), though
56 histopathological and immunological changes exist in infected animals (Netski et al.
57 1999; Lehmer et al. 2007). Within host populations, transmission of SNV is predicted to
58 occur through aggressive interactions. However, this hypothesis is based on the
59 correlation between scarring and SNV infection documented in numerous studies (Boone
60 et al. 1998; Mills et al. 1999; Douglass et al. 2001; Calisher et al. 2007). Transmission
61 has not been directly observed under natural or laboratory conditions and the increased
62 scarring observed in infected individuals could occur after infection, as suggested for
63 other Hantaviruses (Klein et al. 2004). For SNV to spread among deer mice through
64 aggressive encounters, an uninfected deer mouse must first encounter and then
65 aggressively interact with an infected deer mouse. Therefore, those deer mice that exhibit
66 behaviours that increase the probability of intraspecific encounters and/or display more
67 aggressive behaviour should have a higher probability of being infected with SNV.

68

69 The primary goal of this research was to test the hypothesis that infected animals
70 exhibit a suite of behaviours more likely to result in an infection than the population at
71 large. To that end, we observed deer mouse behaviour in a natural setting. Studying
72 behaviour in the wild is a logistical challenge, but it is necessary because behaviours are
73 known to change when wild animals are brought into laboratory settings (Calisi and
74 Bentley 2009). We used a novel mouse surveillance system to observe deer mouse
75 behaviour unadulterated by human presence. We predicted that deer mice infected with
76 SNV would engage more frequently in behaviours that increased the probability of
77 intraspecific encounters and transmission than uninfected deer mice. We defined these
78 behaviours as “risky” with respect to SNV infection. We also predicted that SNV positive
79 deer mice would be mostly heavier, scarred, and reproductive males.

80

81 METHODS

82

83 *Deer Mouse Sampling*

84

85 Our study site was located in the Great Basin Desert of central Utah (Juab county) on
86 lands administered by the US Department of Agriculture and the Bureau of Land
87 Management (Certificate of Registration #1COLL5194, Division of Wildlife Resources,
88 Utah Department of Natural Resources). Vegetation consisted predominately of big
89 sagebrush (*Artemisia tridentata*) and Utah juniper (*Juniperus osteosperma*). Observations
90 were conducted in May, July and September of 2009 and 2010 for a total of 6 observation
91 events.

92 Deer mice were trapped using a web sampling design that consisted of 148 traps over
93 3.14 ha (Mills et al. 1995). The Sherman folding live-traps (3 x 3.5 x 9") contained
94 peanut butter and oats and polyester fiberfill for bedding. Traps were opened at dusk and
95 checked each morning for three consecutive nights. Captures were identified to species
96 and the physical characteristics that were collected included mass, sex, reproductive
97 status and presence of scars. One blood sample ca. 0.2 ml was taken retro-orbitally from
98 deer mice upon each initial capture of each trapping visit. A drop of 0.5% proparacaine
99 hydrochloride ophthalmic solution (©Bausch & Lomb Incorporated) was added to the
100 eye as directed to minimize possible pain associated with collecting the blood sample.
101 Blood samples were immediately placed on dry ice until they could be transferred to an -
102 80°C freezer. Blood samples were tested for IgG antibodies to SNV by an enzyme-linked
103 immunosorbent assay (ELISA; Feldmann et al. 1993). Because viremia is brief in deer
104 mice infected with SNV (Botten et al. 2000; Botten et al. 2003) and because deer mice
105 produce virus-specific antibodies to SNV for life after initial infection (Botten et al.
106 2000), ELISA is the standard method of testing for SNV infection. Finally, each rodent
107 was marked with a passive integrated transponder tag (PIT; TX1400ST, BioMark, Inc.,
108 Boise, ID) injected subcutaneously between the scapulae with a sterile 12-gauge needle.
109 The tags were 12 mm in length, were encased in glass to prevent tissue irritation, and
110 weighed approximately 0.06 g (approximately 0.003% of the weight of our captured
111 mice), making alteration of behaviour unlikely. After processing, animals were released
112 at the point of capture. This research complied with the Institutional Animal Care and
113 Use Committee of the University of Utah (IACUC no. 0802012) and the ASAB/ABS

114 Guidelines for the Use of Animals in Research. Additionally, all workers followed
115 guidelines for working with animals potentially infected with SNV (Mills et al. 1995).

116

117 *Deer Mouse Surveillance*

118

119 After the three nights of deer mouse sampling, traps were removed and within the
120 same area nine camera stations were installed in a three by three grid with stations 50 m
121 apart. Camera stations included an infrared camera (MESSOA, Model SCR351-HN1,
122 Chino, CA) mounted 1 m above ground on a pole. Cameras were attached by above
123 ground cables to a centrally located computer, which was powered by a generator (EU
124 1000, Honda, Alpharetta, GA). The cameras recorded four images per second, and were
125 focused on a 30 cm diameter foraging tray that contained 2 L of sand with 3 g of millet
126 seed. The size and amount of the seed is comparable to that found naturally in sagebrush
127 habitats (Christ and Friese 1993; Allen and Novak 2008) and the rodents had to actively
128 forage in the sand for the seed. Therefore, we consider behaviour on foraging trays to
129 represent normal deer mouse behaviour. Additionally, seed remained in the trays in the
130 morning, suggesting alternate food resources were available to the mice. A foam ring
131 encircled each tray, and acted as a ramp to the tray. Under each tray we placed a PIT
132 antenna connected to a data logger (FS2001FT-ISO, Biomark, Inc., Boise, ID) powered
133 by a 12 V battery. The data loggers recorded the PIT numbers of any deer mice visiting
134 the foraging trays or the immediate vicinity with a time stamp, so that arrival and
135 departure times could be estimated. The loggers can record multiple animals
136 simultaneously. Half of the foraging trays were placed in a position out in the open with

137 no sagebrush cover overhead. These trays were more visible and offered fewer escape
138 options and therefore were termed “exposed”. The other half of the trays were placed
139 under sagebrush cover and termed “protected”. The trays were alternated each evening
140 between an exposed and a protected position (< 2 m apart). Foraging trays were opened,
141 and cameras and loggers collected data, each evening from dusk until shortly after dawn
142 for the four nights immediately following trapping. In the morning, remaining seed in the
143 foraging trays was sifted from the sand, measured, and replaced with a new 3 g of seed.
144 Each tray was covered with a plastic lid until dusk. The video footage and data from the
145 loggers were integrated with software from TimeScience™ (Salt Lake City, UT) to
146 coordinate the identity and the behaviour of the individual with its physical
147 characteristics and infection status.

148

149 *Behaviour*

150

151 The behaviour of each animal observed on trays was categorized as either foraging or
152 an interaction. Foraging was defined as any time an animal spent on a tray alone.
153 Interactions involved more than one animal on or near a tray at a time. We observed five
154 types of interactions: fighting, chasing, avoiding, sharing and allogrooming. Fighting
155 included any aggressive contact between two animals, whereas chasing was aggressive
156 pursuit of one mouse by another without any contact observed. Avoiding included a deer
157 mouse leaving the camera’s view when in the presence of another deer mouse, or a deer
158 mouse entering a foraging tray within 10 s of another deer mouse leaving the tray,
159 presumably waiting outside of the camera’s view until the occupant of the tray left.

160 Sharing was defined as two deer mice foraging on a tray at once, and allogrooming was
161 any non-aggressive contact.

162

163 We were interested in behaviours that increased the probability of intraspecific
164 encounters as well as aggressive behaviours and termed them “risky” with respect to
165 SNV infection. We measured a total of five behaviours: aggressive interactions, total
166 time spent on the foraging trays, an index measuring time spent on exposed trays, a tray x
167 night index, and distance traveled (Table 1). Aggressive interactions were defined as
168 fighting and chasing. We considered exposed tray time to be a risky behaviour in terms of
169 pathogen transmission, as our previous work documented an increased number of
170 intraspecific encounters on exposed trays. Indeed, during this study, we found
171 significantly more encounters (all interactions except avoidance) per time spent on
172 exposed trays than protected trays (Chi-squared proportion test: 0.0015 vs. 0.0009, $p=$
173 0.023). The exposed tray index ($[\text{exposed time}/\text{total time}] * \text{exposed time}$) takes into
174 account both the proportion of time and actual time deer mice spent on exposed trays. We
175 also created a tray x night index to account for the small number of both trays (9) and
176 nights (4) available during each surveillance period. Tray x night is thus a measure of the
177 number of different trays visited by a deer mouse over four nights multiplied by the
178 number of nights the mouse was seen on trays. We calculated the minimum distance
179 traveled by following the path of a deer mouse from tray to tray over the course of each
180 night, assuming that the more distance a deer mouse traveled, the more likely it would
181 encounter another deer mouse. The first tray visited each night received a value of 1 m.
182 All subsequent trays visited received the shortest linear distance from the previous tray. If

183 an animal visited the same tray several times consecutively, each visit received a value of
184 1 m because leaving and returning to an antenna's range required at least this distance.
185 Thus, these are probably quite conservative estimates. Each of the behaviours were
186 totaled for each mouse for each four-day surveillance period.

187

188 We were unable to use repeated measures design because not all individuals were
189 observed during all observation periods. In fact, the majority (79%) of the 63 deer mice
190 were observed in only one sampling period. Ten deer mice were observed in two
191 sampling periods while three were observed in three sampling periods. Infection status
192 did not change across sampling periods for any of the multi-captured deer mice. To
193 account for pseudoreplication in these deer mice, each behaviour was averaged, meaning
194 each deer mouse is represented only once in the statistical analyses. Behaviours were
195 compared between infected and uninfected deer mice using a Student's t-test.

196

197 *Risk Analyses*

198

199 Deer mice were then individually analyzed for risky behaviour using principle
200 components analysis (PCA). PCA is a way to analyze many likely correlated variables
201 (i.e., behaviours) at once. It reduces the observed variables into a smaller number of
202 principal components (artificial variables) that account for the variance in the observed
203 variables. We used the scores given to each deer mouse from PC1 to assign each deer
204 mouse a risk status of either bold or shy. Four of the five behaviours (total time, exposed
205 tray index, tray x night index, and distance) were first normalized using a logarithmic

206 transformation.

207

208 We examined the relationship between SNV status, risk status and physical
209 characteristics using logistic regression with binomial errors and the logit link function.

210 The physical characteristics were sex, reproductive status, scarring, and mass.

211 Reproductive status was based on males having abdominal testes and females having a

212 perforate vagina, being pregnant or lactating. Risk status and most physical

213 characteristics did not change for most multi-captured deer mice between trapping

214 seasons. However, mass did fluctuate and was therefore averaged. Additionally, five of

215 the 13 multi-captured deer mice changed from not scarred to scarred across trapping

216 seasons- they were categorized as scarred in the statistical analyses. The model was

217 simplified using stepwise (backward) elimination based on analysis of deviance and chi-

218 squared statistics. All analyses were performed in R (R Development Core Team 2006)

219 and were considered statistically significant if $P \leq 0.05$.

220

221 RESULTS

222

223 In total, we marked 228 deer mice with PIT tags, plus 102 other rodents (*Perognathus*

224 *parvus* and *Reithrodontomys megalotis*). We observed 63 of the tagged deer mice on

225 foraging trays, with overall SNV prevalence of 30% (19/63). Due to generator failure,

226 observation time totaled 1000 hours. Tagged deer mice were on the trays a total of 61

227 hours, mostly foraging alone. We observed 62 interactions between two deer mice of

228 known infection status. The largest percentage of interactions was aggressive (39%: Fig.

229 1), followed by avoiding (27.5%), sharing (27.5%), and allogrooming (6%).

230

231 *Behaviour*

232

233 Infected deer mice engaged to a greater extent in behaviours deemed risky in terms of
234 pathogen transmission than uninfected deer mice (Table 1). Specifically, they spent 2.9x
235 more time on the foraging trays, had a 2.8x higher exposed tray index, had more than 2x
236 the tray x night index and traveled almost 2.2 times farther than uninfected deer mice (t_{61}
237 > 2.44 , $P < 0.016$ for all). Additionally, infected deer mice were involved in 5.4 times the
238 number of aggressive interactions compared to uninfected deer mice ($t_{63} > 2.12$, $P <$
239 0.038 for all).

240

241 *Risk Analyses*

242

243 PC1 accounted for 63% of the variation in risky behaviours and thus was the only PC
244 we evaluated. For PC1, each deer mouse was given a single value that was a
245 combination of the contributions from each of the five behaviours (Table 2). While PC1
246 retained all five behaviours, the tray x night index was not a significant contributor. We
247 used PC1 to categorize deer mice into bold and shy categories. Twenty deer mice
248 (31.7%) were categorized as bold ($> \frac{1}{2}$ standard deviation above average). All other deer
249 mice ($n=43$) were categorized as shy (62.3%).

250

251 Behaviour, physical characteristics and their interactions were used to predict which

252 deer mice were most likely to be SNV positive. In the final model, bold behaviour was
253 the only predictor of positive SNV status (odds ratio=5.35, 95% confidence interval =
254 0.53-2.89, $P=0.005$). Bold deer mice were three times more likely to be SNV positive
255 than shy deer mice (55% vs. 18.6%). Sex, reproductive status, scarring, mass and all
256 interactions that had sufficient data to be assessed did not improve the fit of the model
257 and were therefore excluded.

258

259 DISCUSSION

260

261 Deer mice appear to forage solitarily. Of the time we observed deer mice on the
262 foraging trays, < 1% of the time involved two mice interacting. Furthermore, 27.5% of
263 the observed interactions involved deer mice avoiding one another (Fig. 1). When deer
264 mice did interact, almost 40% of interactions were aggressive (fighting and chasing).
265 Although non-aggressive interactions (sharing and allogrooming) were observed, most of
266 these interactions involved the same two juvenile individuals, as estimated from mass and
267 coat coloration, which we presumed to be littermates.

268

269 In our study, deer mice infected with SNV exhibited a different suite of behaviours
270 than uninfected deer mice by engaging in risky behaviours more frequently. We defined
271 risky behaviours as those that would increase the likelihood of encountering other deer
272 mice as well as aggressive behaviour. Such behaviour would in turn increase the
273 probability of a pathogen transmission event (Keesing et al. 2006). The behaviours we
274 considered risky are likely part of a behavioural syndrome, which is a suite of correlated

275 behaviours (Sih et al. 2004a). The behaviours that were correlated in this study were
276 total time on the trays, exposed tray index, distance traveled and aggressive interactions.
277 Behavioural syndromes have been found in several taxa, where individuals exhibit a bold
278 or shy behavioural phenotype. (Wilson et al. 1994; Coleman and Wilson 1998; Wilson
279 1998). Other syndromes, for example proactive vs. reactive, have also been suggested
280 (Koolhaus et al 1999; Malmkvist and Hansen 2002). Many ecological and evolutionary
281 processes are known to be affected by behavioural syndromes (Sih et al. 2004b), among
282 them susceptibility to parasitism (Barber and Dingemanse 2010; Boyer et al 2010). In our
283 study, the higher infection prevalence in bold compared to shy deer mice (55% vs.
284 18.6%) can be explained by their behaviour, which showed increased encounter
285 probability and aggressiveness.

286

287 There are two opposing explanations for the observed behavioural differences seen in
288 this study. The first posits that infection causes changes in behaviour. Directly altering
289 the host's behaviour to the benefit of the pathogen is known as adaptive manipulation
290 (Brown 2005; Thomas et al. 2005). For example, some parasites with complex life cycles
291 appear to cause the intermediate host to behave in such a way as to facilitate predation by
292 the definitive host (Lafferty and Morris 1996; Berdoy et al. 2000; Luong et al. 2011).
293 Pathogens that are not trophically transmitted through intermediate hosts, as in the
294 previous examples, can also cause behavioural changes. Rabies virus enters the central
295 nervous system and often makes the host uncharacteristically aggressive (Klein 2003;
296 <http://www.cdc.gov/rabies>). This aggression, along with virus present in the saliva,
297 directly promotes pathogen transmission. Behaviour can also be passively (indirectly)

298 manipulated by the pathogen (Milinski 1990). For instance, if there is a metabolic cost of
299 infection (Lochmiller and Deerenberg 2000; Demas 2004), infected individuals might
300 engage in riskier behaviours to acquire food. Or if a pathogen decreases the life
301 expectancy of the host, then the terminal investment hypothesis predicts a host should
302 invest more in current reproduction than in survival and future reproduction (Clutton-
303 Brock 1984).

304

305 Alternatively, infection could be the result of existing behavioural differences. The
306 20/80 rule states that host heterogeneities cause a small percentage of the host population,
307 approximately 20%, to be responsible for a majority of transmission events (Woolhouse
308 et al. 1997). This rule holds for several pathogens that appear to be transmitted by a
309 small, behaviourally distinct subset of the population (May and Anderson 1987; Dye and
310 Gay 2003; Lloyd-Smith et al. 2005; Clay et al. 2009; Boyer et al. 2010). We modeled
311 SNV status as a function of behaviour and physical characteristics and found relatively
312 more SNV positive individuals in bold vs. shy deer mice (55% vs. 18.6% respectively).
313 Contrary to our prediction, sex, reproductive status, scarring and mass did not influence
314 SNV status. Mass is often used as a surrogate for age (Fairborn 1977), with juveniles <
315 14 g, sub-adults between 14 and 17 g, and adults > 17g (Douglass et al. 2001). Within the
316 bold group, mass ranged from 11.2-28.7 g and the age distribution was similar to that of
317 the entire captured deer mouse population (5% juveniles, 30% subadults, and 65%
318 adults), implying risky behaviours were not associated with any particular age class.

319

320 The hypotheses that certain behaviours are the cause or consequence of infection are

321 not mutually exclusive. Risky behaviour can increase the probability of encountering
322 infection, followed by the pathogen causing increases in risky behaviour to promote its
323 transmission (Barber and Dingemans 2010). Our findings that infected deer mice
324 engaged in risky behaviour could be interpreted as a cause or consequence of SNV
325 infection, or both. To tease apart the hypotheses would require comparing behaviour in
326 the same mice before and after infection. However seroconversions are rare events that
327 are difficult to document let alone obtain a reasonable sample size for statistical analysis.
328 For example, over two years time, we observed only one deer mouse that seroconverted
329 (1.6%). Other studies have also documented that observations of seroconversions are rare
330 even with much more frequent trapping (Douglass et al. 2007). To observe a reasonable
331 sample size of individuals before and after a seroconversion would require a sampling
332 effort that is orders of magnitude beyond the 1000 hrs recorded in this study. Large
333 outdoor enclosures may be a feasible approach for testing this hypothesis and would
334 allow experimental manipulation in a semi-natural setting. Alternatively, we would
335 suggest two modifications to our methods for future studies. First, given that deer mice
336 live on average only 71 days in the wild (Adler et al. 2008), more frequent trapping might
337 allow higher recapture rates than our 20%. Second, more camera stations would likely
338 result in a higher percentage of tagged deer mice visiting foraging trays than we obtained.

339

340 We cannot definitively answer the question as to whether SNV infection is the cause
341 or consequence of risky behaviour. However, the finding that 58% of our infected deer
342 mice were bold means that 42% of the infected deer mice were *not* bold. This large
343 percentage of SNV positive shy deer mice is difficult to explain if infection causes risky

344 behaviour, i.e. we would expect a much lower percentage of positive and shy deer mice.
345 It is possible that many of our deer mice were in early stages of infection and their
346 behaviour had not yet changed. However, this is highly unlikely given the method that is
347 used to determine SNV status. Our ELISA tests for IgG antibodies, which are only
348 detectable around three weeks after initial infection (Botten et al. 2000). During this
349 time, SNV viral N antigen becomes disseminated into various tissues of infected deer
350 mice. Thus, when deer mice test positive by our ELISA, it seems probable that any
351 behavioural effect of virus should have taken effect. Furthermore, Botten et al. (2000)
352 found no consistent histopathological changes associated with infection, and viral antigen
353 was rarely found in the brain suggesting that SNV infection is not altering behaviour
354 directly. Moreover, there was no difference in mass or reproductive status between
355 infected and uninfected deer mice in our study, indicating that indirect manipulation by
356 SNV is also likely. The findings do not rule out SNV causing behavioural changes.
357 However, we believe a more likely scenario is that risky behaviour increases the
358 probability of SNV transmission, leading to high prevalence in the bold group. Not all
359 bold deer mice are infected, because naïve individuals, some of whom are bold, are added
360 to the population through birth. Furthermore, deer mice infected with SNV may be
361 infectious only intermittently and the virus is inefficiently transmitted (Botten et al.
362 2002), such that even if an encounter and aggressive interaction take place, transmission
363 may not occur. At the same time, some of the shy deer mice are infected (18.6%) due to
364 the probability that they will encounter and interact with bold, and therefore likely
365 infected, deer mice.
366

367 To our knowledge, this is the first study to directly observe behaviour of rodents with
368 respect to infection status in their natural environment. With our unique surveillance
369 system, we were able to document rodent behaviours unadulterated by the presence of
370 human observers or a laboratory setting. We found that infected individuals behave
371 differently than uninfected individuals, due to the strong association between SNV
372 seropositivity and risky behaviour. Our data show the usefulness of using behaviour to
373 understand zoonotic pathogen transmission dynamics. A substantial proportion of
374 emerging infectious diseases, and a majority of emerging viruses, are hosted by rodents
375 (Woolhouse and Gowtage-Sequeria 2005), making this is an important group in which to
376 understand the role of behaviour in transmission dynamics. However, rodents are
377 especially difficult to observe in nature, largely because they are small, quick and often
378 nocturnal. Understanding behaviours that results in transmission of zoonotic pathogens
379 could lead to new strategies to reduce exposure and/or transmission to humans, novel
380 means by which to target host population-level control, and a clearer understanding of the
381 causes underlying global emergence of zoonoses.

382

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395

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552

553 **Fig 1.** Observed interactions of deer mice (*Peromyscus maniculatus*, n=63) while on
 554 foraging trays. The total number of interactions of two deer mice of known infection
 555 status was 62 from 1000 hours of video.

556

557 **Table 1**

558 Means \pm the standard error and Student's t-test or results for risky behaviours between
 559 deer mice infected or uninfected with SNV. Means based on four-night surveillance
 560 period.

behaviour	infected (n=19)	uninfected (n=44)	<i>t</i>	<i>p</i> - value
total tray time (s)	3799 \pm 1235	1056 \pm 221	3.26	0.002
exposed tray index (s)	979 \pm 352	264 \pm 66	2.97	0.004
tray x night	13.3 \pm 2.5	6.25 \pm 0.9	3.34	0.001
distance (m)	647 \pm 153	233 \pm 59	3.09	0.003
aggressive interactions	1.67 \pm 0.8	0.31 \pm 0.17	2.31	0.033

561

562 **Table 2**

563 Principal component analysis loadings on PC 1 for the five behaviours deemed risky in
 564 terms of pathogen acquisition. The bolded behaviours are those that made a major
 565 contribution to PC1.

Behavioural variables	Component 1
total tray time	-0.449
exposed tray index	-0.529
tray x night index	-0.160
distance traveled	-0.427
aggressive interactions	-0.558
total proportion of variance	0.632

566

567