11-2013

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

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

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

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

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

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

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

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

METHODS

Deer Mouse Sampling

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

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

Deer Mouse Surveillance

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

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

Behaviour

The behaviour of each animal observed on trays was categorized as either foraging or an interaction. Foraging was defined as any time an animal spent on a tray alone. Interactions involved more than one animal on or near a tray at a time. We observed five types of interactions: fighting, chasing, avoiding, sharing and allogrooming. Fighting included any aggressive contact between two animals, whereas chasing was aggressive pursuit of one mouse by another without any contact observed. Avoiding included a deer mouse leaving the camera’s view when in the presence of another deer mouse, or a deer mouse entering a foraging tray within 10 s of another deer mouse leaving the tray, 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 ([exposed time/total time]*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

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

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

Risk Analyses

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

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

Behaviour

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

Risk Analyses

PC1 accounted for 63% of the variation in risky behaviours and thus was the only PC we evaluated. For PC1, each deer mouse was given a single value that was a combination of the contributions from each of the five behaviours (Table 2). While PC1 retained all five behaviours, the tray x night index was not a significant contributor. We used PC1 to categorize deer mice into bold and shy categories. Twenty deer mice (31.7%) were categorized as bold (> ½ standard deviation above average). All other deer mice (n=43) were categorized as shy (62.3%).

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

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

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

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

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

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

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

ACKNOWLEDGMENTS

Our deepest gratitude goes to Dylan Taylor for his hard work, dedication and good nature throughout this project. Many thanks to Craig Gritzen, Patrice Kurnath, Kevin Kohl, Sean Laverty, Jael Malenke, Johanna Varner and Todd Zolka for their help and humor in the field and lab. Many others assisted in the field and their contribution was invaluable. We thank the editor and reviewers for their thoughtful suggestions. The research was

supported by an NSF-NIH grant (EF-EID 032999) to M. D. D and a Microbial Pathogenesis Training Grant Award (T32AI055434) to L.D. from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health

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

Table 1

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

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

Table 2

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