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 4 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 did uninfected deer mice. 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 were 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.

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 & 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 & 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 (severe adult respiratory syndrome) and HIV (May & Anderson 1987; Dye & 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 et al. 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 (Nichol et al. 1993; Childs et al. 1994) 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), although 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

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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 does 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 & 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 would 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 U.S. Department of Agriculture and the Bureau of Land Management (Certificate of Registration No. 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 six observation events.

Rodents were trapped using a web sampling design that consisted of 148 traps over 3.14 ha (Mills et al. 1995). The Sherman folding live-traps (7.6 × 8.9 × 22.9 cm) contained peanut butter and oats and polyester fibrefill for bedding. Traps were opened at dusk and checked each morning for 3 consecutive nights. We identified captures to species and collected data on physical characteristics that included mass, sex, reproductive status and presence of scars.

A blood sample was collected retro-orbitally from all captures upon initial capture of each trapping event (i.e. a rodent was bled at most once every 8 weeks). The blood sample measured 0.1–0.2 ml, or no more than 1% of the rodent's body weight (10–30 g), which is the maximum amount of blood that can be safely withdrawn (web. jhu.edu/animalcare/procedures/retro-orbital.html). A drop of 0.5% proparacaine hydrochloride ophthalmic solution (Bausch & Lomb) was added to the eye prior to bleeding to minimize possible pain associated with collecting the blood sample. Rodents were monitored until blood flow from the retro-orbital sinus had ceased and again at the time of release. Retro-orbital bleeding is the standard method of blood collection in hantavirus studies because it leaves no external wound, is a rapid method of blood collection (approximately 30 s), thus minimizing stress and discomfort to the animal, and produces a high-quality blood sample necessary

for SNV testing (http://oacu.od.nih.gov/ARAC/documents/Rodent_Bleeding). Researchers performing the retro-orbital bleeding during this study were trained and experienced in the method. The only adverse effect we observed was that 4 out of the 228 (1.7%) captured deer mice appeared to have a nonfunctioning eye on the side that we bled, which we assumed was caused by the retro-orbital bleeding. All four of these deer mice were recaptured during at least one subsequent season of trapping, leading us to believe they were still able to defend their territory and acquire food.

Blood samples were immediately placed on dry ice until they could be transferred to an −80 °C freezer. Blood samples were tested for IgG antibodies to SNV by an enzyme-linked immunosorbent assay (ELISA; Feldmann et al. 1993). Because viremia is brief in deer mice infected with SNV (Botten et al. 2000, 2003) and because deer mice produce virus-specific antibodies to SNV for life after initial infection (Botten et al. 2000), ELISA is the standard method of testing for SNV infection.

Finally, each rodent was marked with a passive integrated transponder tag (PIT; TX1400ST, BioMark, Inc., Boise, ID, U.S.A.) injected subcutaneously between the scapulae with a sterile 12-gauge needle. Since the tag was placed just under the skin, no anesthetic was used. The tags were 12 mm in length, were encased in glass to prevent tissue irritation, and weighed approximately 0.06 g (approximately 0.2–0.6% of the weight of any capture), making alteration of behaviour unlikely. Upon recapture, the most common problem we found with tagged rodents was that the tags had come out of approximately 10% of our captures. Less often (<5%), the tag had migrated to a rump or lateral position. Recapture rates of tagged rodents were similar to recapture rates of untagged rodents (approximately 30%) and no adverse effects were observed in tagged rodents, suggesting tagging did not negatively impact them. Given this, and because deer mice live on average only 71 days in the wild (Adler et al. 2008) and that tag removal would have entailed invasive techniques, PIT tags were left in rodents at the end of the study. After processing, animals were released at the point of capture. This research complied with the Institutional Animal Care and 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 3 nights of deer mouse sampling, we removed traps and installed nine camera stations within the same area in a 3 × 3 grid with stations 50 m apart. Camera stations included an infrared camera (MESSOA, Model SCR351-HN1, Chino, CA, U.S.A.) 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, U.S.A.). The cameras recorded four images per second and were focused on a 30 cm diameter foraging tray that contained 2 litres 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 & Friese 1993; Allen & Nowak 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 4 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 Time-Science™ (Salt Lake City, UT, U.S.A.) 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 either as foraging or as 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. Sharing was defined as two deer mice foraging on a tray at once, and allogrooming was any nonaggressive contact.

We were interested in behaviours that increased the probability of intraspecific encounters as well as aggressive behaviours and termed them 'risky' with respect to SNV infection. We measured a total of five behaviours: aggressive interactions, total time spent on the foraging trays, an index measuring time spent on exposed trays, a tray × night index, and distance travelled (Table 1). Aggressive interactions were defined as fighting and chasing. We considered exposed tray time to be a risky behaviour in terms of pathogen transmission, as our previous work documented an increased number of intraspecific encounters on exposed trays. Indeed, during this study, we found significantly more encounters (all interactions except avoidance) per time spent on exposed trays than on protected trays (chi-square proportion test: 0.0015 versus 0.0009, $P = 0.023$). The exposed tray index ((exposed time/total time) × exposed time) takes into account both the proportion of time and the actual time that deer mice spent on exposed trays. We also created a tray × night index to account for the small number of both trays (9) and nights (4) available during each surveillance period. Tray × night is thus a measure of the number of different trays visited by a deer mouse over 4 nights multiplied by the number of nights the mouse was seen on trays. We calculated the minimum distance travelled by following the path of a deer mouse from tray to tray over the course of each night, assuming that the more distance a deer mouse travelled, the more likely it would encounter another deer mouse. The first tray visited each night received a value of 1 m. 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. All behaviours were totalled for each mouse for each 4-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 a single 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 multicaptured 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 analysed for risky behaviour using principal components analysis (PCA). PCA is a way to analyse 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 × night index, and distance) were first normalized using a logarithmic transformation.

We examined the relationship between SNV status, risk status and physical characteristics using logistic regression with binomial errors and the logit link function. The physical characteristics were sex, reproductive status, scarring and mass. Reproductive status was based on males having abdominal testes and females having a perforate vagina, being pregnant or lactating. Risk status and most physical characteristics did not change for most multicaptured deer mice between trapping seasons. However, mass did fluctuate and was therefore averaged. Additionally, five of the 13 multicaptured deer mice changed from not scarred to scarred across trapping seasons; these individuals were categorized as scarred in the statistical analyses. The model was simplified using stepwise (backward) elimination based on analysis of deviance and chi-square statistics. All analyses were performed in R (R Development Core Team 2006) and were considered statistically significant if $P \leq 0.05$.

RESULTS

In total, we marked 228 deer mice with PIT tags, plus 102 other rodents (*Perognathus parvus* and *Reithrodontomys megalotis*). We observed 63 (28%) of the tagged deer mice on foraging trays, which was similar to our recapture rate of 30%, with overall SNV prevalence of 30% (19/63). Mass is often used as a surrogate for age (Fairbairn 1977), with juveniles <14 g, subadults between 14 and 17 g and adults >17 g (Douglass et al. 2001). Of the 63 tagged deer mice, three were juveniles, 20 were subadults and 40 were adults, and 40% were female (25/63); both age and sex distributions were similar to what we found in the overall population. Because of generator failure, observation time totalled 1000 h. Tagged deer

Table 1
Means ± SEs and Student's *t* test results for risky behaviours between deer mice infected or uninfected with Sin Nombre virus (SNV)

Behaviour	Infected (N=19)	Uninfected (N=44)	t	P
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×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

Means are based on a 4-night surveillance period.
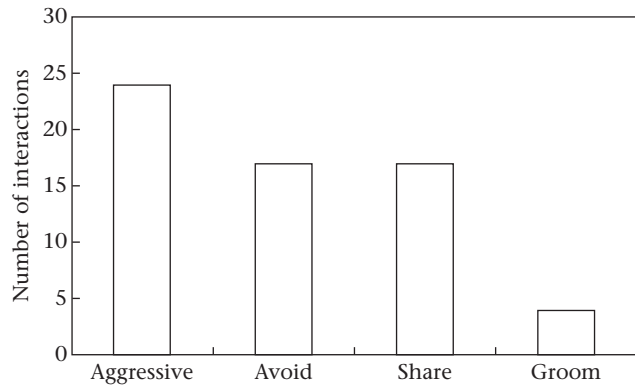
Figure 1. Observed interactions of deer mice, *Peromyscus maniculatus* ($N = 63$), while on foraging trays. Total number of interactions of two deer mice of known infection status was 62, based on 1000 h of video.

mice were on the trays a total of 61 h, mostly foraging alone. We observed 62 interactions between two deer mice of 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 did uninfected deer mice (Table 1). Specifically, they spent $2.9\times$ more time on the foraging trays, had a $2.8\times$ higher exposed tray index, had more than $2\times$ the tray $\times$ night index and travelled 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 $\times$ 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 (>0.5 SD 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 deer mice were most likely to be SNV positive. In the final model, bold behaviour was the only predictor of positive SNV status (odds ratio = 5.35, 95% confidence interval = 0.53–2.89, $P = 0.005$). Bold deer mice were three times

Table 2
Principal component analysis loadings on PC1 for the five behaviours deemed risky in terms of pathogen acquisition

Behavioural variables	PC1
Total tray time	**−0.449**
Exposed tray index	**−0.529**
Tray×night index	−0.160
Distance travelled	**−0.427**
Aggressive interactions	**-0.558**
Total proportion of variance	0.632

Behaviours in bold made a major contribution to PC1.

more likely to be SNV positive than were shy deer mice (55% versus 18.6%). Sex, reproductive status, scarring, mass and all interactions that had sufficient data to be assessed did not improve the fit of the model and were therefore excluded.

DISCUSSION

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

In our study, deer mice infected with SNV exhibited a different suite of behaviours than uninfected deer mice by engaging in risky behaviours more frequently. We defined risky behaviours as those that would increase the likelihood of encountering other deer mice as well as aggressive behaviour. Such behaviour would in turn increase the probability of a pathogen transmission event (Keesing et al. 2006). The behaviours we 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 travelled 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 & Wilson 1998; Wilson 1998). Other syndromes, for example proactive versus reactive, have also been suggested (Koolhaas et al. 1999; Malmkvist & 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 & Dingemanse 2010; Boyer et al. 2010). In our study, the higher infection prevalence in bold compared to shy deer mice (55% versus 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 & 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) manipulated by the pathogen (Milinski 1990). For instance, if there is a metabolic cost of infection (Lochmiller & Deerenberg 2000; Demas 2004), infected individuals might engage in riskier behaviours to acquire food. Or, if a pathogen decreases the life expectancy of the host, then the terminal investment hypothesis predicts that a host should invest more in current reproduction than in survival and future reproduction (Clutton-Brock 1984).

Alternatively, infection could be the result of existing behavioural differences. The 20/80 rule states that host heterogeneities cause a small percentage of the host population, approximately 20%, to be responsible for a majority of transmission events

(Woolhouse et al. 1997). This rule holds for several pathogens that appear to be transmitted by a small, behaviourally distinct subset of the population (May & Anderson 1987; Dye & Gay 2003; Lloyd-Smith et al. 2005; Clay et al. 2009; Boyer et al. 2010). We modelled SNV status as a function of behaviour and physical characteristics and found relatively more SNV positive individuals in bold versus shy deer mice (55% versus 18.6%, respectively). Contrary to our prediction, sex, reproductive status, scarring and mass did not influence SNV status. Within the bold group, mass ranged from 11.2 to 28.7 g and the age distribution was similar to that of the entire captured deer mouse population (5% juveniles, 30% subadults and 65% adults), implying risky behaviours were not associated with any particular age class.

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 & 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 2 years, 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 h recorded in this study. Large outdoor enclosures may be a feasible approach for testing this hypothesis and would allow experimental manipulation in a seminatural 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 used to determine SNV status. Our ELISA tests for IgG antibodies, which are only detectable about 3 weeks after initial infection (Botten et 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 not 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 & 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 result 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.

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