Animal Behaviour 86 (2013) 911–916

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

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

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ARTICLE INFO

Article history:
Received 20 March 2013
Initial acceptance 22 April 2013
Final acceptance 22 July 2013
Available online 12 September 2013
MS. number: A13-00252R

Keywords:
aggressive interaction
deer mice
disease ecology
disease transmission
hantavirus
Peromyscus maniculatus
risky behaviour
zoonotic disease

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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0003-3472/$38.00
http://dx.doi.org/10.1016/j.anbehav.2013.08.003

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 ($7.6 \times 8.9 \times 22.9$ 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 $-80\,^{\circ}$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