Published in final edited form as:

Brain Behav Immun. 2006 March ; 20(2): 182–190. doi:10.1016/j.bbi.2005.06.003.

Social status does not predict responses to Seoul virus infection or reproductive success among male Norway rats

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Abstract

Trade-offs exist among life history strategies that are used to increase survival and reproduction; such that, males that engage in more competitive behaviors may be more susceptible to infection. Hantaviruses are transmitted horizontally between rodents through the passage of virus in saliva during wounding and male rodents are more likely to be infected with hantaviruses than females. To determine whether a trade-off exists between dominance and susceptibility to Seoul virus infection, male Long Evans rats were group housed (3/cage) with a female rat and aggressive and subordinate behaviors were monitored during a 10 day group housing condition. After behavioral testing, males were individually housed, inoculated with Seoul virus, and blood, saliva, and fecal samples were collected. Dominant males initiated more aggressive encounters than subordinate males. Dominant and subordinate males, however, had similar steroid hormone concentrations, anti-Seoul virus IgG responses, and weight gain over the course of infection. A similar proportion of dominant and subordinate males shed virus in saliva and feces during infection. Using microsatellite DNA markers paternity was assigned to pups derived during the group housing period. In contrast to our initial hypothesis, dominant and subordinate males sired a similar percentage of pups. Taken together, host social status may not predict reproductive success or susceptibility to hantaviruses in rodent reservoir populations.

Keywords

Aggression; Corticosterone; Dominance; Hantavirus; Host–parasite coevolution; Life history strategies; Paternity

1. Introduction

Pathogens can create constraints on host populations by limiting survival and reproduction. In response, hosts have evolved adaptations that enable them to avoid infection or to reduce their susceptibility to infection. Presumably, engaging in behaviors that limit exposure to pathogens or mounting immune responses to reduce susceptibility to pathogens requires

metabolic resources that might otherwise be used for other biological processes, such as growth, maintenance of secondary sex characteristics, and reproduction (Barnard and Behnke, 2001; Sheldon and Verhulst, 1996; Zuk and Stoehr, 2002). Thus, trade-offs exist among life history strategies that influence investment in survival and reproduction. Studies in mice illustrate that trade-offs exist between engaging in competitive behaviors (that presumably increase mating opportunities and territorial resources) and susceptibility to infection. Specifically, dominant male mice that are more likely to engage in successful aggressive encounters are more susceptible to infection with nematodes, such as *Heligmosomoides polygyrus*, protozoa, including *Babesia microti*, and viruses, such as herpes simplex than lower rank, less aggressive males (Barnard et al., 1993, 1998; Padgett et al., 1998). The trade-off between competitive abilities and susceptibility to infection is likely mediated by changes in steroid hormone concentrations as well as available metabolic resources (Folstad and Karter, 1992; Sheldon and Verhulst, 1996).

Alternatively, lower ranked males may be more susceptible to infection than dominant males. Several studies illustrate that subordinate males experience an elevated stress response toward antagonistic interactions with dominant individuals that increases their susceptibility to infection. For example, subordinate male cynomolgus monkeys are more likely to develop infection following inoculation with influenza virus than are dominant males (Cohen et al., 1997). Similarly, subordinate mice exhibit immunosuppression and are more susceptible to infection with viruses, such as Moloney virus, and parasites, including *Trypanosoma cruzi*, than their dominant counterparts (Bartolomucci et al., 2001; de Groot et al., 2002; Ebbesen et al., 1991; Raab et al., 1986; Schuster and Schaub, 2001). Reduced immunocompetence among subordinate males may be caused by elevated corticosterone concentrations in response to antagonistic interactions (Blanchard et al., 1993, 1995; Raab et al., 1986; Sapolsky, 2005).

Hantaviruses are negative sense RNA viruses (Family: *Bunyaviridae*) that are primarily maintained by rodents and are found throughout the world. Once infected, reservoir rodents remain persistently infected, which may be caused by changes in the regulation of virus replication, virus induced immunosuppression, or virus evasion of host immune responses (Meyer and Schmaljohn, 2000). Hantaviruses are directly transmitted and are hypothesized to be propagated by inhalation of aerosolized virus in excrement and passage of virus in saliva during aggressive encounters (Glass et al., 1988; Hinson et al., 2004; Lee et al., 1986; Nuzum et al., 1988). Among host reservoir species, including brush mice (*Peromyscus boylii*), deer mice (*P. maniculatus*), western harvest mice (*Reithrodontomys megalotis*), bank voles (*Clethrionomys glareolus*), cotton rats (*Sigmodon hispidus*), and to a lesser extent, Norway rats (*Rattus norvegicus*), adult males are more likely to be infected with hantaviruses than females (Bernshtein et al., 1999; Childs et al., 1987, 1994; Glass et al., 1998; Mills et al., 1998; Weigler et al., 1996). Laboratory studies of Seoul virus infection (i.e., the naturally occurring hantavirus in Norway rats) illustrate that, following inoculation, females are better able to control virus replication in target tissues (e.g., lungs) and shed virus for a shorter duration of time than males (Klein et al., 2000, 2001; Klein et al., 2004a). Sex differences in hantavirus infection may be due to differences in susceptibility to

infection, that are mediated by endocrine-immune interactions (Klein et al., 2000, 2002a,b; Klein et al. 2004a).

In addition to being more susceptible to infection, male Norway rats are more likely to engage in behaviors, such as aggression, that may increase the likelihood of contact with infectious agents (Hinson et al., 2004). Hantaviruses are shed in the saliva of infected animals and virus shedding and seroconversion are correlated with high frequency of wounding among natural populations of Norway rats suggesting that transmission occurs from infected rats biting uninfected individuals (Glass et al., 1988; Hinson et al., 2004). The precise relationship between hantavirus infection and aggressive behavior remains elusive. In several host-pathogen systems, pathogens can manipulate the proximate mechanisms that mediate the expression of behaviors, such as aggression, presumably to facilitate transmission (Klein, 2003; Moore, 2002). Laboratory studies of male Norway rats infected with Seoul virus reveal that during the onset of the persistent phase of infection (i.e., 30 days after inoculation with Seoul virus) males spend more time engaged in aggression during resident-intruder tests than either uninfected males or males tested during the acute phase of infection (i.e., 15 days after inoculation.) (Klein et al., 2004b). Males that engage in aggression for a longer duration of time have more virus present in lung, kidney, and testis than do males that are less aggressive (Klein et al., 2004b). Although certain individuals may be more likely to engage in behaviors that increase the probability of infection, these data suggest that infection with hanta-viruses can alter social behavior in the host.

Whether infection with hantaviruses occurs because certain individuals within a population are more likely to come in contact with infectious virus or whether certain individuals are predisposed to be more susceptible to infection with hantaviruses has not been elucidated. The primary aim of this study was to determine whether trade-offs exist between dominance and susceptibility to Seoul virus infection in Norway rats. Dominance is associated with increased body size, increased resource acquisition, and elevated reproductive success at the cost of increased susceptibility to infectious diseases (Blanchard et al., 1995; Davis, 1953; Hausfater and Watson, 1976; McKean and Nunney, 2001). If dominance is associated with higher reproductive status, then dominant male rats should sire more offspring than subordinate males. If an increased propensity to engage in competitive behaviors, like aggression, increases susceptibility to infection, then dominant males should exhibit lower immune responses and shed virus for a longer duration of time than subordinate males. Conversely, the stress of antagonistic interactions may be elevated in subordinate males and may lead to a reduced ability to control virus replication (Sapolsky, 2005); thus, subordinate males may shed virus for a longer duration of time than dominant males.

2. Methods

2.1. Animals

Out-bred adult (70 days of age) male ($n = 42$) and female ($n = 14$) Long Evans rats (*Rattus norvegicus*) were purchased from Charles River Laboratories, Raleigh, NC, and were from separate litters. Rats were housed in polypropylene cages with filter bonnets in a Biosafety Level 3 animal facility. Animals were provided food and water ad lib and housed with a light dark cycle of 14:10 with lights off at 15:00 h EST. All animal procedures were

approved by Johns Hopkins Animal Care and Use Committee (Protocol Number RA01H466) and Johns Hopkins Office of Health, Safety, and the Environment (A9902030103).

2.2. Procedure

2.2.1. Housing conditions—Rats were weighed and housed individually upon arrival. After 2 days of acclimation, animals were anesthetized with an intramuscular (im) injection of a Keta-mine HCl (80 mg/kg)/xylazine (6 mg/kg) (Phoenix Pharmaceutical, St. Joseph, MO) cocktail, given ear markings, and numbered on their flanks using Clariol Nice and Easy hair dye. A 0.5 cm tail snip was obtained to isolate genomic DNA for microsatellite analyses (see below). All animals were given 2 weeks to recover from the tail snip before beginning behavioral testing. Using microsatellite analyses, animals were assigned to groups of three males and one female, all with distinct genomic loci at each of the sites listed (see below).

2.2.2. Behavioral testing—Animals were group housed in standard cages ($48.3 \times 26.7 \times 20.3$ cm) for 10 consecutive days and behavioral observations were recorded for 1 h at the onset of the dark cycle (under red lights) on days 2, 4, 6, 8, and 10 of the group housing condition (Barnard et al., 1993; Blanchard et al., 1995; Klein et al., 2004b). All behavioral observations were video taped for subsequent analysis. On day 10 of the group housing, each female was removed from their group cage, weighed, and individually housed. At this time, an age and weight matched intruder male was placed in each cage and behaviors were videotaped under red lights. All intruder males were pair-housed prior to behavioral testing. Intruder tests were conducted because previous studies illustrate that once a dominance hierarchy has been formed among rats, dominant males tend to attack intruder males more often than subordinate males (Blanchard et al., 1977, 1988). This is a well-established experimental model for assessing social status in rats (Blanchard et al., 1977, 1995). Intruder males were removed immediately after behavioral testing on Day 10 and resident males were individually housed.

Videotapes were scored using the following behavioral classifications: offensive behaviors (chase, tail rattle, aggressive upright, and offensive bite), defensive behaviors (flee, freeze, crouch, and supine posture), social exploratory behaviors (allogroom and anogenital sniff), and maintenance behaviors (sleep, eat/drink, and self-groom) (Blanchard et al., 1993, 1977; Klein et al., 2004b). The duration and frequency were recorded for each behavior. Because several cages of males did not exhibit sufficient offensive and defensive behaviors during the 1 h tests on days 2–8 of the housing condition, dominance rank was assigned to males based on the number of offensive behavior initiated relative to the number of defensive behaviors exhibited during the intruder male test on day 10 of the housing condition. To assess the reproductive output of each male, females were monitored for pregnancy and, within 24 h after birth, pups were sexed and weighed and the kidneys were removed for isolation of genomic DNA and paternity analysis (see below).

2.2.3. Infection—After the group housing period, male rats were individually housed, weighed, and bled from the retro-orbital sinus. Male rats were then inoculated in the

intraperitoneal cavity with 10^4 plaque forming units of Seoul virus strain SR-11 suspended in 0.2 cc of minimum essential Eagle's Medium (with Earle's salts; Mediatech Cellgro, Herndon, VA). On days 10, 15, 20, 30, and 40 post-inoculation (pi), male rats were anaesthetized with Halothane (Halocarbon Laboratories, River Edge, NJ) mixed 1:4 with mineral oil, weighed, and blood, saliva, and fecal samples were collected. On Day 40 pi, after saliva, feces, and blood were collected, animals were killed and reproductive organs, adrenal glands, spleen, and thymus samples were collected.

2.2.4. Microsatellite analyses—Genomic DNA was isolated from tail samples collected from adult male and female rats and kidneys collected from pups using the QIAamp DNA Mini kit following the manufacturer's protocol (Qiagen, Valencia, CA). Nine microsatellite markers (D1Cebr3, D1Cebr4, D2Cebr1, D3Cebr3, D4Cebr2, D4Cebr3, D5Cebr1, D6Cebr1, and D20Cebr1) were used to amplify loci on chromosomes 1, 2, 3, 4, 5, 6, and 20, respectively (Giraudeau et al., 1999). Primers were custom synthesized and the forward primers were labeled with fluorescent markers 6FAM, HEX, or NED (Applied Biosystems, Foster City, CA). One hundred microgram of genomic DNA were amplified in a 50 μ l reaction volume with 0.25 nM of each primer set and reagents from the AmpliTaq Kit (Applied Biosystems). Reactions were amplified for one cycle at 95 °C for 90 s and 30 cycles of 95 °C for 40 s, 55 °C for 40 s, and 70 °C for 2 min, followed by 1 h at 70 °C. PCR products were stored at 4 °C and run within 48 h.

After PCR amplification, products from each rat were multiplexed in a 96-well plate by adding 0.4–0.7 μ l of PCR product for each locus per well containing HiDi formamide and GeneScan-500 ROX standard at a ratio of 1:20 (Applied Biosystems). Samples were processed on the Applied Biosystems 3100-Avant using a 50 cm capillary and the manufacturer's fragment analysis protocol (Applied Biosystems). Raw microsatellite data were analyzed using GeneScan Analysis 3.7 and genotypes were assigned using Genotyper 3.7 (Applied Bio-systems).

2.2.5. Enzyme-linked immunosorbent assay (ELISA)—Microtiter plates were coated overnight at 4 °C with gamma irradiated Vero E6 cells infected with Seoul virus or gamma irradiated uninfected Vero E6 cells diluted in carbonate buffer. Thawed plasma samples, as well as positive and negative control samples, were diluted 1:100 in PBS–Tween (PBS–T) with 2% fetal bovine serum (FBS) and added in duplicate to antigen-coated wells containing either infected or uninfected Vero E6 cells. The plates were incubated at 37 °C for 1 h and secondary antibody (alkaline phosphatase-conjugated anti-rat IgG (H+L), Kirkegaard and Perry Laboratories, Gaithersburg, MD) diluted in PBS with 2% FBS was added. The plates were incubated for 1 h at 37 °C and substrate buffer (0.5 mg/ml *p*-nitrophenyl phosphate diluted in diethanolamine substrate buffer) was added. The enzyme–substrate reaction was terminated, the optical density (OD) was measured at 405 nm, and the average OD for each set of uninfected Vero E6 duplicates was subtracted from the average OD for each set of infected Vero E6 duplicates. Samples were considered positive if the average adjusted OD was ≥ 0.100 (Klein et al., 2000, 2001). To minimize intra- and inter-plate variability, the average adjusted OD for each sample was expressed as a percentage of its plate positive control OD for statistical analyses.

2.2.6. Steroid enzyme immunoassay—Testosterone and corticosterone concentrations were measured in sera samples collected 1 day after the 10 day group housing period. Steroid hormones were extracted from sera using diethyl ether and concentrations were measured using the manufacturer's protocol for the enzyme immunoassay (EIA) (Cayman Chemicals, Ann Arbor, MI).

2.2.7. RNA isolation and reverse transcription (RT)-PCR—RNA was isolated from saliva and feces using Trizol LS and the manufacturer's protocol as described previously (Invitrogen, Carlsbad, CA) (Klein et al., 2000, 2001). First-strand synthesis cDNA was prepared using random hexamers and the Invitrogen Superscript III First Strand Synthesis reagents and the manufacturer's protocol (Invitrogen). The positive control was Seoul virus RNA isolated from our virus stock (strain SR-11) and the negative control was DEPC-treated water included in the cDNA syntheses and in both primary and secondary amplifications. Outer primers were used to amplify a 280-bp sequence of the Seoul virus genome in a 100 μ l reaction mixture containing 20 μ l of the cDNA. The nested 176-bp sequence was amplified in a 100 μ l reaction mixture containing the nested primers and 2 μ l of the first DNA amplification product as described previously (Klein et al., 2000, 2001, 2002a). Primary and secondary reactions each were amplified for one cycle at 94 °C for 3 min and 40 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 60 s, followed by incubation for 10 min at 72 °C. The nested PCR products were electrophoresed on a 2.5% sodium borate agarose gel (Faster Better Media LLC, Hunt Valley, MD) (Brody and Kern, 2004), stained with ethidium bromide, and examined for bands at 176 bp.

2.2.8. Statistical analyses—Because minimal offensive and defensive behaviors were observed on days 2–8 of the group housing condition, only behavioral observations made on day 10 of the group housing condition (i.e., when an intruder male was placed into each cage of three resident males) were used for data analyses (Blanchard et al., 1977). Males were ranked based on their attack ratio (i.e., the number of offensive behaviors/the number of defensive behaviors) (Barnard et al., 1993). The duration and frequency of each behavior, steroid hormone concentrations, organ masses, and litter data were compared among males using one-way ANOVAs. Body mass and antibody responses were analyzed using mixed ANOVAs with one within subjects variable (days post-inoculation) and one between subjects variable (treatment group). The proportion of animals shedding virus in saliva and feces was compared among groups using χ^2 analyses. In cases where the data violated the assumptions of a normal distribution, nonparametric statistics were used. Significant interactions were further analyzed using the Tukey or Dunn method for pairwise multiple comparisons. Mean differences were considered statistically significant if $p < .05$.

3. Results

3.1. Behavior

Males ranked as dominant (i.e., dominance rank = 1) had a higher attack ratio than males ranked as subordinates (i.e., dominance rank = 2 or 3) ($F(2,39) = 5.76, p < .05$; Fig. 1). The duration and frequency of offensive behaviors were higher among dominant than subordinate males ($H(2) = 14.58, p < .05$ and $F(2,39) = 3.37, p < .05$, respectively). In

contrast, the duration and frequency of defensive behaviors did not differ between dominant and subordinate males ($p > .05$ in each case; Table 1). Dominant and subordinate male did not differ in either the duration or frequency of social investigatory (i.e., anogenital sniffing and grooming) or maintenance (i.e., eating, drinking, and sleeping) behaviors exhibited during the intruder tests ($p > .05$ in each case; Table 1).

3.2. Hormones, body mass, and organ masses

Testosterone concentrations measured after the 10 group housing period were similar among dominant (Rank 1 = $2.01 \pm .68$ ng/ml) and subordinate (Rank 2 = 2.12 ± 1.01 ng/ml, Rank 3 = $2.29 \pm .42$ ng/ml) males ($p > .05$). Corticosterone concentrations also did not differ significantly among dominant (Rank 1 = 180.1 ± 21.0 ng/ml) and subordinate (Rank 2 = 182.8 ± 12.2 ng/ml, Rank 3 = 200.8 ± 17.9 ng/ml) males ($p > .05$). Weight gain over the course of the 10 day group housing condition did not differ among dominant and subordinate males ($F(1, 39) = 64.27$, $p < .05$; Fig. 2). Dominant and subordinate males gained similar amounts of weight over the course of infection (i.e., from Day 0 to Day 40 pi) ($F(5, 185) = 657.94$, $p < .05$; Fig. 2). Neither relative nor absolute testes, epididymides, seminal vesicle, adrenal gland, thymus gland, or spleen masses differed between dominant and subordinate males ($p > .05$ in each case; Table 2).

3.3. Infection status

For both dominant and subordinate males, anti-Seoul virus IgG responses increased over the course of infection with antibody responses peaking 30 and 40 days after inoculation ($F(5, 190) = 85.39$, $p < .05$; Fig. 3). Antibody responses against Seoul virus did not differ between dominant and subordinate males ($p > .05$; Fig. 3). A similar proportion of dominant and subordinate males shed virus in saliva and feces over the course of infection ($p > .05$; Table 3).

3.4. Paternity analyses

To assess whether dominant males sire more offspring than subordinate males, microsatellite genotype comparisons were used to assign paternity to offspring derived from the group housing period. Eight out of the 14 females sired litters resulting in 102 pups that were used for data analyses. Comparison of unique paternal variation at the selected microsatellite loci was successfully used to assign paternity to 80.4% (82/102) of the sired pups. For the remaining unmatched pups, odds ratios (4:1 or higher) based on the nine microsatellite loci were used to assign paternity to 80.0% (16/20) of the pups (Ribble, 1991). Paternity could not accurately be assigned to the remaining 3.9% (4/102) of the pups originating from three separate litters. Dominant and subordinate males sired a similar percentage of pups within each litter ($p > .05$; Table 4). The average birth weight, anogenital distance, and the male:female ratio of pups sired by dominant and subordinate males also did not differ ($p > .05$; Table 4).

4. Discussion

Mounting an immune response and minimizing pathogen replication can be metabolically costly to the host (Demas, 2004; Demas et al., 1997; Hanssen et al., 2004; McKean and

Nunney, 2001; Raberg et al., 2000). Thus, host defenses should represent the optimal allocation of resources to reduce susceptibility to infection while adequately maintaining other costly functions, such as reproduction. Investment in reproduction can increase susceptibility to some parasites (Festa-Bianchet, 1989; Gustafsson et al., 1994; Nordling et al., 1998; Norris et al., 1994; Richner et al., 1995). Most studies characterizing the trade-off between reproductive effort and immunity use either nonreplicating pathogens or antigens (Demas et al., 1997; Hanssen et al., 2004; Raberg et al., 2000) or manipulate reproductive effort and examine subsequent effects on responses to infection (Nordling et al., 1998; Richner et al., 1995). To date, whether a trade-off exists between reproductive success (as measured by the quantity and quality of sired offspring) and susceptibility to a naturally occurring, endemic pathogen has not been reported. In the present study, social status did not predict susceptibility to hantavirus infection or reproductive success among Norway rats. Dominant and subordinate males were equally likely to sire pups and shed virus, and had similar antibody responses and weight gain over the course of infection.

In contrast to our initial hypothesis, dominant and subordinate males sired an equivalent proportion of pups within each litter that were of similar quality, in terms of body mass and reproductive development (i.e., AGD) at the time of birth. Dominant and subordinate males also sired a similar proportion of male and female pups. Among rats, dominant males are more likely to copulate with an estrous female than are subordinate males (Thor and Flannelly, 1979). After the first few ejaculations, however, the dominant male advantage is lost and successful mating attempts can be achieved by subordinate males (Barfield and Thomas, 1986). In multi-male mating tests, the second male that copulates with a female typically has a siring advantage based on DNA fingerprinting analyses (Shimmin et al., 1995). Thus, reproductive success may be determined by the outcome of sperm competition in the reproductive tract of the female. Females gain genetic benefits (e.g., genetic diversity) from mating with multiple males. Long Evans rats are outbred and increased genetic diversity in the offspring may drive the evolution of females mating with multiple males as has been previously demonstrated in mice and birds (Ehman and Scott, 2004; Foerster et al., 2003). Recent data from mice infected with *H. polygyrus* illustrate that neither dominance nor infection status predict reproductive success, based on microsatellite analysis, in mice (Ehman and Scott, 2004).

Consistent with the results of the present study, social status does not predict responses to helminth parasites in either baboons or mice (Barnard et al., 1998, 2003; Muller-Graf et al., 1996). Studies in mice illustrate that the variation between dominant and subordinate males is most pronounced when the animals are challenged by exposure to stressors. For example, the stress of social reorganization (i.e., repeatedly removing the dominant male from a social hierarchy) facilitates reactivation of latent herpes virus in mice, with reactivation being more pronounced among dominant than subordinate mice (Padgett et al., 1998). Consequently, exposure to the stress of social reorganization causes increased inflammatory responses and glucocorticoid resistance in subordinate mice (Avitsur et al., 2001). In species for which dominance is attained through aggression and maintained through intimidation (e.g., rats), subordinates typically exhibit increased stressor-induced disease compared with dominant individuals (Sapolsky, 2005). Whether exposure to stressors would differentially impact

dominant and subordinate male rats to affect subsequent responses to Seoul virus infection has not been reported and requires additional investigation.

Behavioral dominance, as determined by aggressive encounters, is the most well-established and reliable mode of determining social status in rodents (Blanchard et al., 1995, 1977, 1988, 2004). Other potential indices of dominance, including reproductive success and responses to infection, did not differ between dominant and subordinate males in the present study. Neither steroid hormone concentrations nor testes or adrenal masses, measured after the 10 day group housing condition, reflected social status in the present study. Reports of differences in steroid hormone concentrations between dominant and subordinate males are inconsistent, in which testosterone concentrations often do not differ between dominant and subordinate males, whereas corticosterone concentrations are elevated by lower social rank (Barnard et al., 1993, 1994, 1998; Blanchard et al., 1995). The increase in corticosterone concentrations with lower social status is most pronounced when changes in concentrations prior to and after group housing are examined (Barnard et al., 1993, 1998). Unfortunately, blood was not collected from male rats prior to group housing in the present study; thus, only post-group housing concentrations were examined and were modestly higher among lower rank males. The robustness of the data from the present study may be limited by the artificial housing condition (i.e., small cages), which may contribute to the lack of aggression observed prior to the intruder male tests. Utilization of a BSL3 pathogen limits our ability to house laboratory rats under more naturalistic conditions, such as in a visible burrow system (Blanchard et al., 1995). For hantaviruses, assessment of wounding and infection status in natural populations of Norway rats may increase our power to detect meaningful differences between dominant and subordinate males.

Hantaviruses have existed for millions of years and have coevolved with specific rodent hosts. Genetically related rodents carry hantaviruses with nearly identical gene and protein sequences and analysis of rodent host mitochondrial and viral gene sequences can be used to reconstruct similar evolutionary trees (Plyusnin and Morzunov, 2001). Seoul virus is the naturally occurring hantavirus that infects Norway rats. Infection of adult rats does not cause pathology and does not appear to alter growth, fertility, or survival in natural populations of male and female Norway rats (Childs et al., 1989; Klein et al., 2002a). Thus, mounting immune responses and reducing replication of hantaviruses may not exert differential metabolic costs between dominant and subordinate individuals in such a highly coevolved host–pathogen system.

Although rodents remain persistently infected, only during a limited period of time are animals shedding virus in saliva, urine, and feces and capable of infecting uninfected individuals (Klein et al., 2000; Lee et al., 1981, 1986). Virus shedding typically occurs 10–30 days pi and is followed by a chronic period of infection that is characterized by active immune responses in the absence of virus shedding (Meyer and Schmaljohn, 2000). The mechanisms mediating virus shedding are not known, but previous data from our laboratory illustrate that male rats shed virus for a longer duration of time than females and this appears to be androgen-dependent (Klein et al., 2002b). In the present study, dominant and subordinate males shed virus for a similar duration of time. A majority of the rats, regardless of rank, were still shedding virus by Day 40 pi. In the present study, only the presence or

absence of viral RNA was assessed; whether social status impacts infectiousness (i.e., the release of infectious virus in excrement and saliva) of male rats requires additional investigation.

Intraspecific transmission of hantaviruses appears to occur through contact with saliva during aggressive encounters (Glass et al., 1988; Hinson et al., 2004). Increased aggressive behavior at the onset of sexual maturity correlates with increased Seoul virus transmission among adult Norway rats (Glass et al., 1988; Hinson et al., 2004). Whether engaging in aggressive behavior increases exposure to hantaviruses (i.e., host-mediated hypothesis) or whether infection increases the propensity to engage in aggression (i.e., parasite-mediated hypothesis) remains unclear. Recent data from our laboratory reveal that male rats that engage in aggressive behavior for a longer duration of time have more Seoul virus present in lung, kidney, and testis tissues than do males that are less aggressive (Klein et al., 2004b). Neither viral RNA nor protein are present in the brains of infected males (Botten et al., 2000; Hinson et al., 2004; Kawamura et al., 1991). Thus, the changes in host aggressive behavior may be caused by elevated virus replication in peripheral target tissues, such as the testes, and may be communicated to the CNS via hormonal signals. Whether this is due to parasite-manipulation of host behavior or is a side-effect of increased virus replication in peripheral tissues requires additional investigation. The data from the present study provide pivotal information about the co-evolution of hantaviruses with their rodent hosts and suggest that the costs of infection may not be substantial in a highly co-evolved system. Whether host social status affects the likelihood of being exposed to Seoul virus remains to be determined.

Acknowledgments

We thank Connie Schmaljohn, Cindy Rossi, and Kristen Spik, at the U.S. Army Medical Research Institute of Infectious Diseases, for providing hantavirus reagents. We also thank Amy Cernetich, Judy Easterbrook, Scott Shone, and Aimee West for technical assistance. Financial support provided by NIH R01 AI 054995 (S.L.K.) and NASA grant NCC5-305 (G.E.G.).

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Fig. 1.

Average ($\pm$ SEM) attack ratio (i.e., the number of offensive behaviors/the number of defensive behaviors) for males ranked as dominant (rank = 1) or subordinate (rank = 2 or 3). Regression analyses were used to assign rank within each cage (i.e., the male with the highest attack ratio within each cage was assigned 1, the male with the next highest attack ratio was assigned 2, and the male with the lowest attack ratio was assigned 3).

Fig. 2. Average ($\pm$ SEM) body mass (g) for males ranked as dominant (rank = 1) or subordinate (rank = 2 or 3) prior to group housing (-10 days post-inoculation) and 0, 10, 15, 20, 30, and 40 days after inoculation with Seoul virus.

Fig. 3. Plasma anti-Seoul virus IgG responses (mean $\pm$ SEM) in male rats based on dominance rank. Blood samples were collected 0, 10, 15, 20, 30, and 40 days following inoculation with Seoul virus. Data are presented as IgG units, in which the mean OD of each test sample was divided by the OD of the positive control sample run on the same microtiter plate.

Table 1

Average ($\pm$ SEM) frequency and duration of defensive, offensive, social, and maintenance behaviors exhibited, based on male dominance rank

Behavior		Dominance rank		
		1	2	3
Offensive	(#)	10.79 $\pm$ 5.01 *	3.00 $\pm$ 0.99	0.36 $\pm$ 0.16
	(s)	48.21 $\pm$ 25.50 *	17.92 $\pm$ 8.75	0.85 $\pm$ 0.52
Defensive	(#)	3.21 $\pm$ 1.59	4.21 $\pm$ 1.75	4.00 $\pm$ 1.29
	(s)	15.92 $\pm$ 9.40	24.28 $\pm$ 12.06	33.64 $\pm$ 20.48
Social investigation	(#)	28.92 $\pm$ 3.17	31.57 $\pm$ 4.38	33.14 $\pm$ 4.04
	(s)	522.2 $\pm$ 68.1	507.9 $\pm$ 69.3	507.1 $\pm$ 58.6
Maintenance	(#)	14.92 $\pm$ 2.38	16.00 $\pm$ 1.81	15.42 $\pm$ 1.51
	(s)	679.1 $\pm$ 100.4	740.3 $\pm$ 87.3	860.7 $\pm$ 112.0

* Asterisk indicates that dominant males (Rank 1) differ significantly from subordinate males (Rank 2 and 3), $p < .05$.

Table 2Average ($\pm$ SEM) absolute organ masses (g) based on male dominance rank

Organ (g)	Dominance rank		
	1	2	3
Testes	3.46 $\pm$ 0.21	3.50 $\pm$ 0.21	3.65 $\pm$ 0.15
Epididymides	1.33 $\pm$ 0.04	1.34 $\pm$ 0.06	1.30 $\pm$ 0.03
Seminal vesicles	0.57 $\pm$ 0.03	0.52 $\pm$ 0.02	0.51 $\pm$ 0.02
Adrenal glands	0.13 $\pm$ 0.01	0.13 $\pm$ 0.01	0.12 $\pm$ 0.01
Spleen	0.69 $\pm$ 0.03	0.65 $\pm$ 0.02	0.69 $\pm$ 0.04
Thymus	0.57 $\pm$ 0.04	0.58 $\pm$ 0.03	0.56 $\pm$ 0.03

Table 3

Proportion of males shedding virus in saliva and feces 10, 15, 20, 30, and 40 days after inoculation with Seoul virus, based on dominance rank

Sample ^a and group	Day post-inoculation				
	10	15	20	30	40
<i>Saliva</i>					
Rank 1	10/14	7/13	7/13	6/13	9/12
Rank 2	11/14	5/12	8/13	7/13	10/13
Rank 3	11/13	8/14	6/13	8/14	7/13
<i>Feces</i>					
Rank 1	13/14	11/14	6/14	11/14	9/13
Rank 2	12/14	9/12	11/13	5/12	8/12
Rank 3	13/13	11/13	8/14	10/14	8/14

^aDiscrepancies in sample sizes were due to insufficient samples.

Table 4Birth statistics (mean $\pm$ SEM) based on male dominance rank

Dependent variable	Dominance rank		
	1	2	3
Proportion of pups sired	29.3 $\pm$ 11	38.2 $\pm$ 8.41	32.4 $\pm$ 8.01
Sex ratio ^a	1.50 $\pm$ 0.10	1.52 $\pm$ 0.08	1.56 $\pm$ 0.08
Birth weight (g)			
Males	6.48 $\pm$ 0.16	6.36 $\pm$ 0.15	6.66 $\pm$ 0.11
Females	6.31 $\pm$ 0.19	6.39 $\pm$ 0.12	6.19 $\pm$ 0.21
Anogenital distance (mm)			
Males	3.08 $\pm$ 0.08	3.17 $\pm$ 0.08	3.12 $\pm$ 0.08
Females	1.61 $\pm$ 0.06	1.47 $\pm$ 0.05	1.54 $\pm$ 0.06

^aSex ratio of pups sired by each male was assessed by arbitrarily assigning 1 = female and 2 = male; thus, a 1:1 female:male ratio = 1.5.