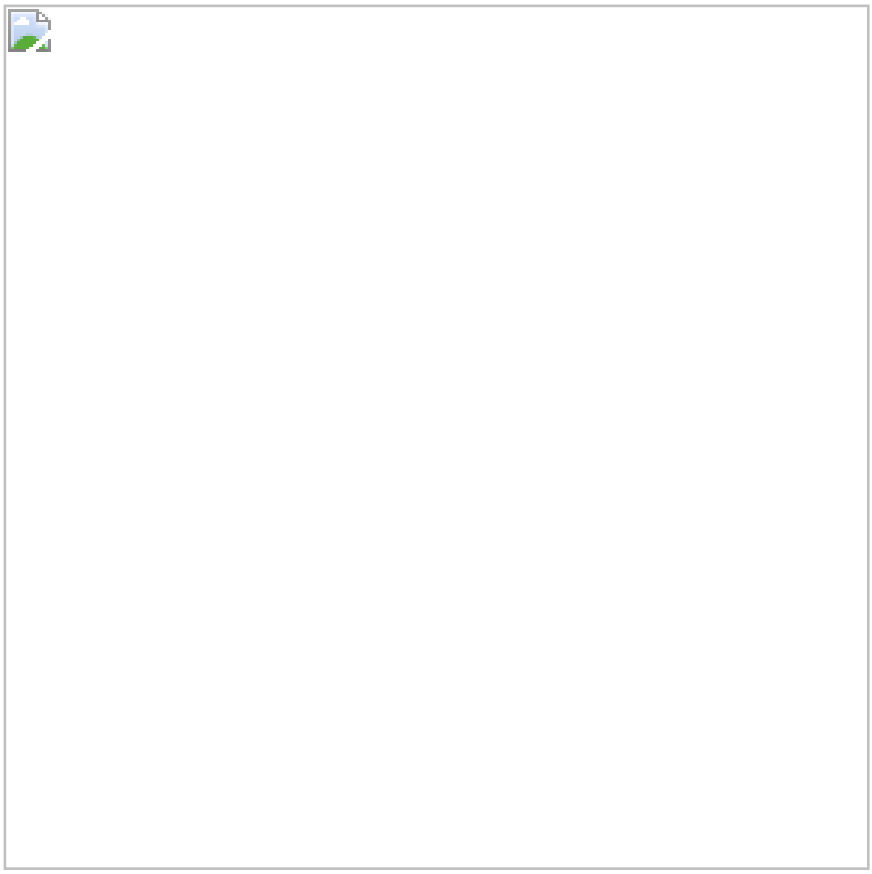


How can hantaviruses kill humans but leave deer mice unaffected?

Authors: McLean, Nellie, Kelly, Ashley, Molinar, Edlin, Ghachu, Joni, Hart, Lacey, et al.

Source: BIOS, 83(3) : 81-89

Published By: Beta Beta Beta Biological Society

URL: https://doi.org/10.1893/0005-3155-83.3.81

Research Article

How can hantaviruses kill humans but leave deer mice unaffected?

An evaluation of the immune response of deer mice to Sin Nombre virus

Nellie McLean, Ashley Kelly, Edlin Molinar, Joni Ghachu, Lacey Hart, Colleen O'Brien, Kimberly Wright, Tony Schountz[1], Cathy Hartney and Erin M. Lehmer

Fort Lewis College, Durango, CO 81301,
[1]University of Northern Colorado, Greeley, CO 80639

Abstract. Sin Nombre virus (SNV) causes hantavirus pulmonary syndrome (HPS) in humans, a disease with high (~36%) mortality. Deer mice (*Peromyscus maniculatus*) are the primary host of SNV and, unlike humans, deer mice infected with SNV have few overt signs of disease. The reasons for such mild infections in deer mice have not been well studied; however, this information may be useful in uncovering therapies that could reduce human HPS mortality. Therefore, the objective of this study was to evaluate the immune response of deer mice to SNV by examining their patterns of white blood cell production. We quantified the number of lymphocytes, eosinophils, basophils, neutrophils, and monocytes produced by wild deer mice in both the early and late stages of SNV infection. Deer mice captured in the early season had greater basophil, lymphocyte, and eosinophil levels compared to deer mice captured in the late season. Conversely, monocyte levels were greater in deer mice captured in the late season. SNV infection status appeared to influence production of both neutrophils and monocytes, with SNV-infected mice having greater neutrophil levels but lower monocyte levels than uninfected mice. Collectively, the results seem to support the notion that immune stressors faced by wild deer mice shift from early to late season, and these differences are reflected by differential leukocyte production that occurs across seasons. Furthermore, our results indicate some potential differences between wild deer mice and lab-bred deer mice, as well as some possible similarities between wild deer mice and humans in their immune responses to SNV infection.

Introduction

Zoonotic diseases are caused by pathogens originating in an animal host that can be transmitted to humans (CDC, 2005). Sin Nombre virus (SNV) is a zoonotic pathogen first identified in 1993 following a hantavirus pulmonary syndrome (HPS) outbreak in the Four Corners region of the southwestern Unites States (Mills, 2005). SNV is a hantavirus; hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) are thought to have evolved in Europe and Asia and are hosted almost exclusively by rodents (Mills et al,. 1999; Botten et al., 2002). At least 30 hantaviruses have been identified worldwide

Correspondence to: lehmer_e@fortlewis.edu
Keywords: immunity

Bios 83(3) 81–89, 2012

and are typically classified as either Old or New World in origin (Mills et al., 1999). In North America, SNV causes nearly all cases of HPS in the United States, and this pathogen is hosted almost exclusively by deer mice (*Peromyscus maniculatus*; Mills et al., 2005).

Although deer mice do not experience increased mortality or decreased fitness from SNV infection, they do produce a measurable immune response to the virus (Schountz et al., 2007). Deer mice produce antibodies that neutralize SNV, but the antibodies produced are insufficient in clearing the viral infection; thus, deer mice remain infected for life with few to no outward signs of disease (Schountz et al., 2007). At the cellular level, viral antigen expression is highest during the first 3-4 weeks of infection, a phase known as the acute stage of infection. Viral antigen expression typically peaks around day 21 and declines thereafter, as deer mice enter the persistent stage of infection (Botten et al., 2003). T cells from acutely infected deer mice typically have increased transcription of proinflammatory cytokines (Easterbrook and Klein, 2008). In contrast, in the persistent stage of infection, deer mice often experience reductions in pro-inflammatory cytokine production, as well as elevations of regulatory responses that suppress pro-inflammatory cytokine production (Schountz et al., 2007; Easterbrook and Klein, 2008). During the chronic stage of infection, SNV enter a period of functional latency, where viral replication is reduced and viral titers are maintained at low levels in the heart, lungs, and brown adipose tissue (Botten et al., 2003). In both acute and persistent stages of infection, deer mice pose a significant threat to human health, as infected deer mice shed virus soon after infection via urine, feces, and saliva and humans can become infected with the SNV if they come into respiratory contact with these shed virus particles (Mills et al., 1999; Botten et al., 2002; Prescott et al., 2007).

In contrast to deer mice, humans have a strong and often fatal immune response to SNV infection (Yates et al., 2002; Easterbrook and Klein, 2008). Humans infected with SNV can develop HPS, a condition that often presents in its earliest stages with flu-like symptoms, which rapidly progress (Yates et al., 2002). In later stages, HPS is characterized by increased permeability of the vascular system, resulting in severe pulmonary edema and respiratory failure (Terajima et al., 2007; Borges et al., 2008; Zaki et al., 1995). At the cellular level, the immune response to SNV is driven in large part by alterations in cytokine production. For example, TNF, a pro-inflammatory cytokine, is thought to be responsible for the increased permeability of endothelial capillaries (Borges et al., 2008). The role of cytokines in the immune response to SNV is inherently tied to leukocyte levels, as leukocytes modulate cytokine production. Leukocytes are classified as neutrophils, basophils, eosinophils, monocytes and lymphocytes, which function in the inflammatory immune response to bacterial or fungal pathogens, inflammation, parasitic pathogens, phagocytosis, and cell mediated immunity, respectively. Not surprisingly, HPS is associated with profound shifts in leukocyte production. For example, patients with severe HPS had substantially elevated lymphocyte counts compared to both uninfected control groups and patients presenting with mild HPS (Koster et al., 2001). Furthermore, a number of researchers have suggested that the human immune response to SNV is both maladaptive and over-reactive because individuals with weaker immune systems or lower lymphocyte levels have the highest HPS survival rates (Kitsutani et al., 1999). Interestingly, in the terminal stage of HPS, the total neutrophil counts of most patients do not differ from uninfected individuals (Koster et al., 2001).

Early identification and medical therapies for HPS have been greatly improved in recent years; however, the mortality rate remains at 36% (CDC). Thus, understanding differences between the immune responses of humans and deer mice to SNV will likely be critical to future advances in treatment of HPS. A number of laboratory studies have monitored the immune response of deer mice to SNV; however, it remains unclear how wild deer mice respond to natural SNV infections. Because humans become infected via indirect contact with wild

deer mice, this lack of information represents a critical knowledge gap. Thus, our objective was to evaluate the immune response of deer mice to SNV by examining their patterns of white blood cell production. Specifically our goals were to compare lymphocyte, eosinophil, basophil, neutrophil, and monocyte production between wild deer mice in both acute and chronic stages of SNV infection.

Materials and Methods

Study sites

Deer mice were collected from 12 separate 10,000 m^2 trapping grids (100 traps distributed across 10, 100-m transects) located within a 65 km radius of Durango, CO. These sites were chosen because of high deer mouse densities and SNV prevalence. Trapping was conducted over a two-year period; early season trapping occurred in June 2009 and late season trapping occurred in July and August of 2010. These sampling periods were selected based on life history traits typical of wild deer mice, in which new SNV infections typically peak in April and May following the peak of reproductive activity (Kuenzi et al., 2005; Lehmer et al., 2010). Based on this assumption, wild deer mice are most likely to be in the acute phase of infection in June and then move into the chronic stage of infection by July. Although these methods do not guarantee the stage of infection of individual deer mice, they provide a high likelihood that our early and late samples contain a large number of mice in the appropriate stage of infection without extensive assays that were beyond the scope and budget of the present study.

Small mammal processing

After capture, small mammals were identified to species, weighed for body mass, and then reproductive condition was identified. Deer mice weighing under 14.0 g were considered juveniles (Lehmer et al., 2010) and were excluded from further processing. Juvenile deer mice receive maternal antibodies during gestation and lactation. This passive immunity persists for up to 4 weeks post weaning. Thus juvenile deer mice may test positive for SNV during this period without having an active SNV infection (Douglass et al., 2007). Approximately 0.3 ml of blood was collected from all adult deer mice via the retroorbital sinus using heparinized microcapillary tubes. A small subsample of blood (0.1 ml) was used to create a monolayer blood smear by placing a drop of blood onto a glass microscope slide and using another glass slide to feather the blood at a 45^0 angle. Blood smears were allowed to dry and then were stored at 20°C for further analyses. The remaining blood was stored on dry ice in the field and later transferred to a -20°C freezer on the Fort Lewis College campus until further assays were run. After processing, rodents were released back to their capture sites. All procedures for trapping and handling small mammals were approved by the IACUC at Fort Lewis College and CDC guidelines for handling rodents potentially infected with SNV were followed.

Leukocyte profiles

Upon returning to the lab at Fort Lewis College, blood smears collected in the field were stained using the Wright 3-step protocol (Rodak et al., 2007). After staining, microscopes at 100x and 400x magnification were used to identify locations on slides where the monolayer smears were ideal for counting individual cells. Then, using oil immersion lenses at 1000x, leukocytes were systematically counted by moving horizontally and vertically across the entire surface of the slide. In this process all leukocytes encountered on a single slide were differentially classified as lymphocytes, eosinophils, basophils, neutrophils, or monocytes. Macrophages were neither encountered nor counted, as these leukocytes are typically differentiated from monocytes to macrophages as they cross the endothelium and are found only in tissues and not typically in whole blood (Chomarat et al., 2000).

SNV antibody detection

Using enzyme-linked immunosorbent assays (ELISA), SNV infection status of captured deer mice was determined. ELISA tests for the

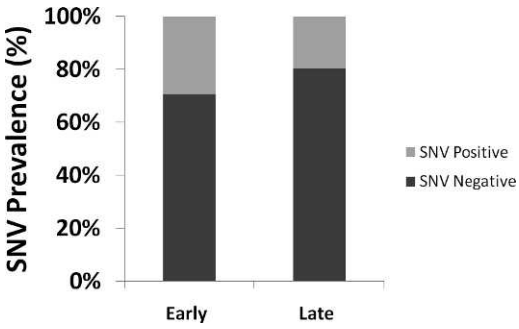


Figure 1. Sin Nombre Virus (SNV) prevalence for deer mouse (*Peromyscus maniculatus*) populations trapped with a 65 km radius of Durango, CO. Trapping was conducted over a two year period; "Early" season occurred in June 2009 and "Late" season trapping occurred in July and August of 2010. In the Early season SNV prevalence was 29.4% (n = 97), while in the late season SNV prevalence was 19.6% (n = 46).

presence of SNV antibodies (IgG) in serum (Otteson et al., 1996). Because deer mice are chronically infected with SNV, they produce antibodies to SNV throughout life. Thus, presence of SNV antibodies in sera is a reliable proxy for determining SNV infection (Schountz et al., 2007; Easterbrook and Klein 2008; Clay et al., 2009). Prior to performing the ELISAs, deer mouse sera were heat inactivated in a laminar flow hood in the BSL-2 laboratory at Fort Lewis College. In this process, whole blood samples were placed in a 55°C water bath for 30 minutes, and then centrifuged to separate serum from whole blood. ELISAs were performed following the protocol described previously by Otteson et al. (1996) and Feldman et al. (1993). Briefly, 96 well plates were coated with recombinant SNV nucleocapsid antigen at 1:400 in phosphate buffered saline (PBS) and stored overnight at 4°C. After washing wells in PBS with 0.5% Tween-20 (PBS-T), heat inactivated serum samples were diluted 1:100 in PBS with 0.5% Tween-20 and 5% skim milk (milk diluent), plated in duplicate and then incubated for 60-120 minutes at 37°C. Goat anti-*Peromyscus leucopus* HRP secondary antibody was diluted to 1:1000 in milk diluent and incubated for 60 minutes at 37°C. After incubation, plates were washed three times in PBS-T and ABTS peroxidase substrate (Kirkegaard and Perry Laboratories) was added to the wells. Plates were incubated for 30 minutes at 37°C to develop the assay and then were read on a Tecan Infinite M200 plate reader. Deer mice were considered seropositive if their optical densities were > 3x negative control sera.

Statistical analysis

Differences in leukocyte levels between infected and uninfected deer mice, as well as between individuals in acute and chronic stages of infection, were measured with Analysis of Variance (ANOVA). In these models, individual deer mice were coded for both SNV infection status and capture season. Leukocyte counts for each individual are the continuous dependent variables and independent factors included year, infection status, and their interaction terms. Differences were considered statistically significant if $P < 0.05$. Pairwise differences of interest were measured with Tukey's HSD ($\alpha = 0.05$). Means are presented as proportions of the total leukocyte count.

Results

SNV prevalence

Over the course of two sampling seasons, we captured 97 unique deer mice (n=97). In 2009, we captured 51 deer mice, including 15 that were infected with SNV and 36 that were uninfected. In 2009, SNV prevalence on our study sites was 29.4% (Figure 1). In 2010, we captured 46 deer mice, including 9 that were infected with SNV and 37 that were uninfected. In 2010, SNV prevalence across our study sites was 19.6% (Figure 1).

Basophil levels

There were no differences in basophil levels between SNV infected and uninfected mice (infected mean = 0.019+ 0.010, uninfected mean = 0.029 + 0.006; $F_{1,93} = 0.648$, $P = 0.423$; Figure 2). However, basophil levels differed between seasons, with deer mice captured in the early season having greater numbers of basophils, compared to mice

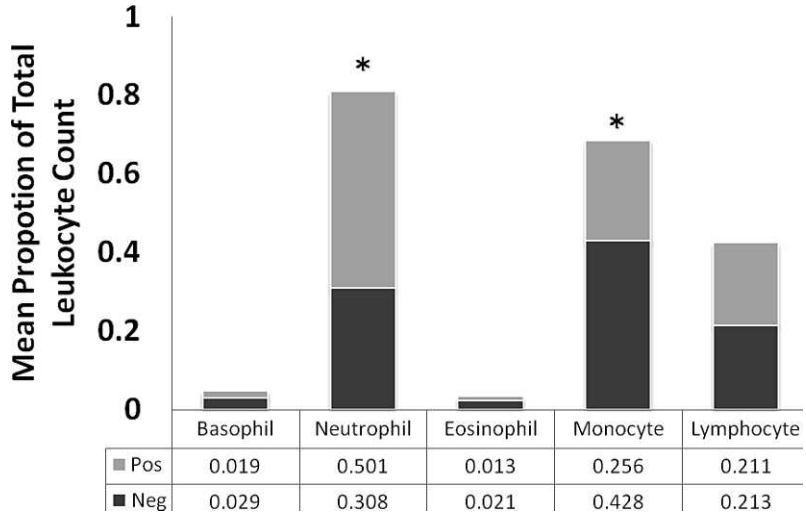


Figure 2. Differential leukocyte profiles for deer mice (*Peromyscus maniculatus)* infected with Sin Nombre virus (n = 24) and uninfected individuals (n = 73) captured in 2009 and 2010. Differences in leukocyte levels between SNV-infected and uninfected were evaluated with ANOVA (α= 0.05).*indicates a significant difference between groups for a particular leukocyte type.

captured in the late season (2009 mean = 0.049 + 0.008, 2010 mean = 0.00 + 0.009; $F_{1,93}$ = 16.510, $P < .000$; Figure 3). There was no significant season x infection interaction ($F_{1,93}$=0.648, P =0.423).

Neutrophil levels

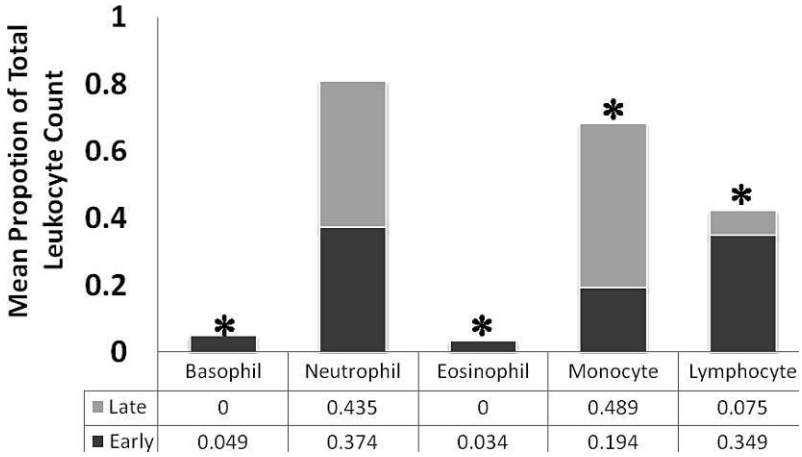
Neutrophil levels were influenced by SNV infection status, as infected deer mice had higher neutrophil counts compared to their uninfected counterparts (uninfected mean = 0.308 + 0.032, infected mean = 0.501 + 0.058; $F_{1,93}$ = 8.581, P = 0.004; Figure 2). Neutrophil levels were not influenced by seasons, as we found no differences in neutrophil counts between deer mice captured in early and late seasons (2009 mean = 0.374 + 0.042, 2010 mean = 0.435 + 0.051; $F_{1,93}$= 0.846, P = 0.360); however, there was a significant season x infection status interaction ($F_{1,93}$= 7.857, P =.006).

Eosinophil levels

There were no differences in eosinophil levels between SNV infected and uninfected mice (uninfected mean = 0.021 + 0.004, infected mean = 0.013 + 0.006; $F_{1,93}$=1.391, P = 0.241; Figure 2). However, eosinophil levels differed between seasons, with deer mice captured in the early season having greater eosinophil counts compared to deer mice captured in the late season (2009 mean = 0.034 + 0.005, 2010 mean = 0.001 + 0.006; $F_{1,93}$=21.395, P = 0.000; Figure 3). There was no significant season x infection interaction ($F_{1,93}$=1.391, P = 0.241).

Monocyte levels

Monocyte levels were influenced by SNV infection status, with uninfected deer mice having greater monocyte counts compared to their SNV-infected counterparts (uninfected mean = 0.428 + 0.030, infected mean = 0.256 + 0.055; $F_{1,93}$ = 7.515, P = 0.007; Figure 2). Monocyte levels also differed between seasons, with deer mice captured in the late season having higher monocyte counts compared to deer mice captured in the early season (2009 mean = 0.194 + 0.040, 2010 mean = 0.489 + 0.048; $F_{1,93}$ = 22.132, P = 0.000; Figure 3). There was a significant season x infection interaction ($F_{1,93}$=22.132, $P <$ 0.001).



	Basophil	Neutrophil	Eosinophil	Monocyte	Lymphocyte
Late	0	0.435	0	0.489	0.075
Early	0.049	0.374	0.034	0.194	0.349

Figure 3. Differential leukocyte profiles for deer mice (*Peromyscus maniculatus)* captured in the Early season (June 2009, n = 97) and Late season (July – August 2010, n = 46). Differences in leukocyte levels between Early and Late seasons were evaluated with ANOVA (α= 0.05).*indicates a significant difference between Early and Late season for a particular leukocyte type.

Lymphocyte levels

There were no differences in lymphocyte levels between SNV infected and uninfected mice (uninfected mean = 0.213 + 0.021, infected mean = 0.211 + 0.038; $F_{1,93}$ = 0.004, P = 0.947; Figure 2). However, lymphocyte counts differed seasonally, with deer mice captured in the early season having greater lymphocyte counts than deer mice captured in the late season (2009 mean = 0.349 + 0.027, 2010 mean = 0.075 + 0.033; $F_{1,93}$ = 40.460, $P < .001$; Figure 3). There was no significant season x infection interaction ($F_{1,93}$= 0.478, P = 0.491).

Discussion

Collectively, our results demonstrate that there are significant differences in leukocyte profiles in deer mice in early and late stages of infection, or between deer mice infected with SNV and their uninfected counterparts. Below we discuss the patterns of leukocyte production we observed in wild deer mice, and the significance of these findings in the context of their immune response to SNV.

SNV prevalence

SNV prevalence in our study populations declined between the first and second years of the study; however, it is important to emphasize that, because the study populations in 2009 and 2010 were comprised of different individuals, these patterns are not reflective of an increased incidence of infection from early to late season. SNV prevalence is highly variable across both temporal and spatial scales and, because deer mice are relatively short lived, study populations that are repeatedly sampled over time often show dramatic fluctuations in SNV prevalence between sampling periods (Lehmer et al., 2008).

Seasonal trends

In our study, we looked to identify trends in seasonality and infection status. We observed similar seasonal patterns of basophil, eosinophil and lymphocyte profiles among deer mice, in that regardless of their SNV infection status, deer mice captured in the early season had greater numbers of these leukocytes compared to deer mice captured in the late season. Conversely, monocyte levels increased in the late season. Neutrophil levels were unchanged across seasons. These results are significant because they suggest that seasonal stressors may have a greater influence on immune system function than does infection with SNV. Because SNV does not cause acute illness in deer mice

(Hjelle and Yates, 2001; Yee et al., 2003), it is possible that deer mice increase fitness by reducing their immune response to this non-lethal pathogen. Indeed, previous studies have described a similar "resistance – tolerance tradeoff" in other wild animals and have suggested that, by reducing the response to non-lethal pathogens, animals increase fitness, as tolerating a mild disease caused by non-lethal pathogens may be less physiologically demanding than the cost of mounting immunological resistance (Råberg et al., 2007; Boots, 2008). Furthermore, other researchers have proposed that offsetting fitness costs of SNV infection in this manner may have promoted the long and relatively stable co-evolutionary history that deer mice have with SNV (Schountz et al., 2007).

Seasonality has been cited as an important modulator of immune system function in wild mammals (Demas and Nelson, 1998a; Demas and Nelson, 1998b; Zhou et al., 2002). Previous lab studies focused on general immunity have shown that male deer mice exposed to shorter day lengths and lower ambient temperatures have increased leukocyte production compared to their counterparts exposed to longer day lengths and higher ambient temperatures (Demas and Nelson, 1998b). Similar studies in Syrian (*Mesocricetus auratus*) and Siberian (*Phodopus sungorous*) hamsters have shown that although photoperiod can affect leukocyte proliferation, activation of these leukocytes is not dependent on photoperiod (Zhou et al., 2002). Because our study focused on the relative abundance of leukocytes in finite blood samples, rather than on total leukocyte counts, we cannot make predictions about overall immunocompetence of deer mice in our study. However, our study design allows us to infer priority in leukocyte production across seasons. Our study took place between the months of June and August, when the photoperiod ranged from 14h 42m in mid-June to 13h and 35m in mid-August, spanning a little over an hour photoperiod difference between the early and late seasons (Weather Underground, Inc., 2011). While this difference in photoperiod may seem small, it represents a shift between two seasons (reproductive and non-reproductive) in the short life (> 6 months) of a deer mouse. In our study, longer photoperiods were associated with deer mice having elevated basophil, lymphocyte, and eosinophil levels, which are generally upregulated in response to allergens, viral infections and parasitic infections, respectively (Tizard, 1984; Gleich and Adolphson, 1986). In contrast, deer mice captured during seasons with shorter photoperiods had substantially elevated monocyte levels, comprising over 48% of the total leucocytes counted. Wild deer mice cope with a number of biotic and abiotic stressors, including shifts in forage quality and availability, as well as energetic costs of thermoregulation, evading predation, exposure to other pathogens and reproduction. Each of these factors imposes demands on the energy budgets of small mammals, which in turn affects their immunocompetence (Klein et al., 2000; Lehmer et al., 2007). Thus, deer mice may balance these energetic demands by allocating resources to immune system function differentially across seasons. Collectively, our results seem to support the notion that immune stressors faced by wild deer mice shift from early to late season, and these differences are reflected by differential leukocyte production that occurs across seasons.

SNV infection status trends

Although we observed no difference in basophil, eosinophil and lymphocyte levels between SNV-infected and uninfected mice, SNV infection did appear to influence monocyte and neutrophil production. Whereas monocyte levels were greater in uninfected deer mice, neutrophil production was greater in SNV-infected mice. These results indicate that the inflammatory immune response of wild deer mice is elevated in response to SNV, as neutrophils initiate a cascade of pro-inflammatory cytokine production, including production of TNF-α. These findings are potentially significant in that they differ from those reported for captive deer mice infected with SNV. Previous lab-based studies have shown that chronically infected reservoirs of hantaviruses suppress inflammatory immune pathways

and inflammatory cytokine production (Schountz et al., 2007; Easterbrook and Klein 2008). In contrast, humans have substantially elevated pro-inflammatory immune responses to SNV, which is often fatal. Specifically, humans infected with SNV often experience marked increases in TNF-α production, which is likely the cause of increased permeability of endothelial capillaries that ultimately results in severe pulmonary edema, the hallmark of HPS (Yates et al., 2002; Borges et al., 2008; Easterbrook and Klein, 2008; Mori et al., 1999). Although measuring cytokine production was beyond the scope of the present study, our results highlight potential differences between the immune responses of wild and lab-bred deer mice to SNV, as well as some potential similarities in the immune response to SNV between wild deer mice and humans. Future studies should more closely evaluate patterns of cytokine production in wild deer mice infected with SNV.

We acknowledge that our study has a number of limitations that are inherent in studying infection in wild mammal populations, including, for example, an inability to pinpoint date of infection and to obtain localized versus peripheral blood samples. However, because wild deer mice are responsible for transmission of SNV to humans, field-based studies provide valuable insight about factors that regulate SNV transmission in the natural environment. Thus, despite these limitations, future studies should continue to examine the immune response of wild deer mice to SNV infection.

Acknowledgments: Research support was provided by the Mountain Studies Institute, Fort Lewis College and the Fort Lewis College Foundation. We thank N. Bourjaily, D. Newbold, M. Ziemke, J. Christensen, Z. Jones, S. Hileman, E. Herring, A. McCarthy, S. Donelan and numerous undergraduate research assistants for help in the field and lab.

Literature Cited

Boots, M. (2008). Fight or learn to live with the consequences. *Trends in Ecology and Evolution* 23, 248–250.

Botten, J., Mirowsk, K., Kusewitt, D., Ye, C., Gottlieb, K., Prescott, J., and Hjelle, B. (2003). Persistent Sin Nombre Virus Infection in the Deer Mouse (*Peromyscus Maniculatus*) Model: Sites of Replication and Strand-Specific Expression. *American Society for Microbiology: Journal of Virology* 2, 1540–1550.

Botten, J., Mirowsky , K., Ye, C., Gottlieb, K., Saavedra, M., Ponce, L., and Hjelle, B. (2002). Shedding and Intercage Transmission of Sin Nombre Hantavirus in the Deer Mouse (*Peromyscus Maniculatus)* Model. *American Society for Microbiology: Journal of Virology* 76, 7587–7594.

Borges, A.A., Campos, G.M., Moreli, M.L., Souza, R.L.M., Saggioro, F.P., Figueirdo, G.G., Livonesi, M.C., and Figueiredo, L.T.M. (2008). Role of Mixed Th1 and Th2 Serum Cytokines on Pathogenesis and Prognosis of Hantavirus Pulmonary Syndrome. *Elsevier Masson SAS. Microbes and Infection* 10, 1150–1157.

Centers for Disease Control and Prevention (2005). "Compendium of Measures to Prevent Disease Associated with Animals in Public Settings, 2005: National Association of State Public Health Veterinarians, Inc. (NASPHV)" Morbidity and Mortality World Report. 54, RR–4.

Chomarat, P., Banchereau, J., Davoust, J. and Palucka, K. (2000). IL-6 switches the Differentiation of Monocytes from Dendritic Cells to Macrophages. *Nature Immunology* 1, 510–514.

Clay, C., Lehmer, E., Previtali, A., Jeor, S., Dearing, D. (2009). Contact Heterogeneity in Deer Mice: Implications for Sin Nombre Virus Transmission. *Proceedings of the Royal Society B 276* (1660), 1305–1312.

Demas, G.E. and Nelson, R.J. (1998a). Short-day Enhancement of Immune Function is Independent of Steroid Hormones in Deer Mice (*Peromyscus Maniculatus)*. *The Journal of Comparative Physiology B* 168, 418–426.

Demas, D.E. and Nelson, R.J. (1998b). Photoperiod, Ambient Temperature, and Food Availability Interact to Affect Reproductive and Immune Function in Adult Male Deer Mice Mouse (*Peromyscus Maniculatus)*. *Journal of Biological Rhythms* 13(3), 94–102.

Douglass, R., Calisher, C., Wagoner, K., and Mills, J. (2007). Sin Nombre Virus Infection of Deer Mice in Montana: Characteristics of Newly Infected Mice, Incidence, and Temporal Pattern of Infection. *Journal of Wildlife Diseases* 43(1), 12–22.

Easterbrook, J.D., and Klein, S. L. 2008. Immunological Mechanisms Mediating Hantavirus Persistence in Rodent Reservoirs. *PLos Pathogens* 4(11), 1–8.

Feldman, H., Sanchez, A., Murozonov, S., Spiropoulou, C.F., Rollin, P.E., Ksiazek, T.G., et al. (1993). Utilization of autopsy RNA for the synthesis of the nucleocapsid antigen of a newly recognized virus associated with hantavirus pulmonary syndrome. *Virus Research* 30, 351–367.

Gleich, G. and Adolphson, C. (1986). The Eosinophilic Leukocyte: Structure and Function. *Journal of Leukocyte Biology* 81, 584–592.

Hjelle B. and Yates T. (2001). The Hantaviruses. In: Smaljohn, C.S. and S.T. Nichol (eds.), Modeling hantavirus maintenance and transmission in rodent communities. Springer-Verlag, Berlin 77–90.

Kitsutani, P., Denton, R., Frita, C., Murray, R., Todd, R., Pape, W., Frampton, J., Young, J., Khan, A., Peters, C., and Ksiazek, T. (1999). Acute Sin Nombre Hantavirus

Infection without Pulmonary Syndrome. United States. *Emerging Infectious Diseases* 5, 701–705.

Klein, S.L., Bird, B.H., and Glass, G.E. (2000). Sex Differences in Seoul Virus Infection are not Related to Adult Sex Steroid Concentrations in Norway Rats. *Journal of Virology* 74, 8213–8217.

Koster, F., Foucar, K., Hjelle, B., Scott, A., Chong, Y., Larson, R. , and Mccabe, M. (2001). Rapid Presumptive Diagnosis of Hantavirus Cardiopulmonary Syndrome by Peripheral Blood Smear. Hematopathology. *American Society of Clinical Pathologists*. *Am J Clin* 116, 665–672.

Kuenzi, A.J., Douglass, R.J., Bond, C.W., Calisher, C.H., and Mills, J.N. (2005). Long-Term Dynamics of Sin Nombre Viral RNA and Antibody in Deer Mice in Montana. *Journal of Wildlife Diseases* 41(3), 473–481.

Lehmer E.M., Clay, C.A., Pierce-Duvet, J., St. Jeor, S., and Dearing, M.D. (2008). Differential regulation of pathogens: the role of habitat disturbance in predicting prevalence of Sin Nombre virus. *Oecologia* 155, 429–439.

Lehmer E.M., Jones, J.D., Bego, M.G., Varner, J.M., Jeor, S.S., Clay, C.A., and Dearing, M.D. (2010). Long-Term Patterns Of Immune Investment By Wild Deer Mice Infected With Sin Nombre Virus. *Physiol Biochem Zoology* 83(5), 847–57.

Lehmer, E., Clay, C., Wilson, E., Jeor, S., Dearing, D. (2007). Differential Resource Allocation in Deer Mice Exposed to Sin Nombre Virus. *Physiological and Biochemical Zoology* 80(5), 514–521.

Mills, J. (2005). Regulation of Rodent-Borne Viruses in the Natural Host: Implications for Human Disease. Archives of Virology. Division of Viral And Rickettsial Diseases, Center For Disease Control And Prevention, Atlanta, GA. Springer-Verlag, 45–75.

Mills, J., Yates, T., Ksiazek, T., Peters, C., and Childs, J. (1999). Long-Term Studies of Hantavirus Reservoir Populations in the Southwestern United States: Rationale, Potential, and Methods. *Emerging Infectious Diseases* 5, 95–101.

Mori, M., Rothman, A.L., Kurane, I., Montoya, J.M., Nolte, K.B., Norman, J.E., Waite, D.C., Koster, F.T., and Ennis, F.A. (1999). High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. *The Journal of Infectious Diseases* 179, 295–302.

Otteson, E.W., Riolo, J., Rowe, J.E., Nichol, S.T., Ksiazek, T.G., Rollin, P.E., and St. Jeor. S.C. (1996). Occurrence of Hantavirus within the Rodent Population of Northeastern California and Nevada. *American Journal of Tropical Medicine and Hygiene* 54, 127–33.

Prescott, J., Hall, P., Bondu-Hawkins, V., Te, C., and Hjelle, B. (2007). Early Innate Immune Responses to Sin Nombre Hantavirus Occur Independently of IFN Regulatory Factor 3, Characterized Pattern Recognition Receptors, and Viral Entry. *J. Immunology* 179, 1796–1802.

Råberg, L., Sim, D., and Read. A. F. (2007). Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. *Science* 318, 812–814.

Rodak, B.F., Fritsma, G.A., and Doig, K. (2007). Chapter 15: Examination of the Peripheral Blood Smear and Correlation with the Complete Blood Count Hematology: Clinical Principals and Applications. (Eds. L. B. Maedel and K. D.) pp 177–181. Saunders, and imprint of Elsevier Inc. St. Louis, Missouri , USA.

Schountz, T., Prescott, J., Cogswell, A., Oko, L., Miroesky-Garcia, K., Galvez, A., and Hjelle, B. (2007). Regulatory T Cell-Like Responses in Deer Mice Persistently Infected with Sin Nombre Virus PNAS 104, 5496–15501.

Terajima, M., Hayasaka, D., Maeda, K, and Ennis, F. (2007). Immunopathogenesis of Hantavirus Pulmonary Syndrome and Hemorrhagic Fever with Renal Syndrome: Do CD8+ T Cells Trigger Capillary Leakage in Viral Hemorrhagic Fevers. *Immuno Lett* 113(2), 117–120.

Tizard, I. (1984). Immunology: An Introduction. 4th edition Texas A & M University. Sanders College Publishing.

Weather Underground, Inc (2011). Accessed March 1, 2011 at http://www.wunderground.com/history/airportfrompws/KDRO/2010/8/15/DailyHistory.html?req_city=NA&req_state=NA&req_statename=NA

Yates, T., Mills, J., Parmenter, C., Ksiazek, T., Parmenter, R., Vande Castle, J., Calisher, C., Nichol, S., Abbot, K., Young, J., Morrison, M., Beaty, B., Dunnum, J., Baker, R., Salazar-Bravo, J., and Perters, C. (2002). The Ecology and Evolutionary History of an Emergent Disease: Hantavirus Pulmonary Syndrome. *Bioscience* 52, 989–998.

Yee, J., Wortman, I.A., Nofchlssey, R.A., Goade, D., Bennett, S.G., Webb, J.P., Irwin, W. and Hjelle, B. (2003). Rapid and Simple Method for Screening Wild Rodents for Antibodies to Sin Nombre Hantavirus. *Journal of Wildlife Diseases* 39, 271–277.

Zaki, S.R., Greer, P.W., Coffield, L.M., Goldsmith, C.S., Nolte, K.B., Foucar, K., Fedderson, R.M., Miller, G.L., Khan, A.S., Rollin, P.E., Ksiazek, T.G., Nichol, S.T., Mahy, B.W.J., and Peters, C.J. (1995). Hantavirus Pulmonary Syndrome: Pathogenesis of an Emerging Infectious Disease. *American Journal of Pathology* 146, 552–579.

Zhou, S., Cagampang, F.R.A., Stirland, J.A., Loudon, A.S.I., and Hopkins, S.J. (2002). Different Photoperiods Affect Proliferation of Lymphocytes but Not Expression of Cellular, Humoral, or Innate Immunity in Hamsters. Journal of Biological Rhythms 17 (5), 392–405.

Received 29 April 2011; accepted 15 November 2011.