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Source: Journal of Wildlife Diseases, 56(3) : 717-720

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/2019-04-104

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DOI: 10.7589/2019-04-104

Journal of Wildlife Diseases, 56(3), 2020, pp. 717–720
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Leukocyte Evaluation of the Free-Ranging Deermouse (*Peromyscus maniculatus*) from Montana, USA

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ABSTRACT: We generated reference ranges for seasonal leukocyte differential counts of the free-ranging deermouse (*Peromyscus maniculatus*) from Montana, US. Blood was collected from the retro-orbital capillary sinus of deermice after topical anesthesia with proparacaine. Although season influenced lymphocyte, neutrophil, and monocyte absolute counts, sex and reproductive status did not.

Hematology is useful for field studies on wildlife health, but for these studies to be successful, we need reference ranges for free-ranging wildlife (Davis et al. 2008). Although studies have evaluated leukocyte counts in laboratory deermice (*Peromyscus maniculatus*; Wiedmeyer et al. 2014), only two have done so in free-ranging deermice (McLean et al. 2012; Orozco 2015). This is problematic because free-ranging wildlife is faced with environmental factors that can influence the immune system, making comparisons between laboratory and free-ranging deermice difficult (Martin et al. 2008). Importantly, studies on free-ranging deermice reported only leukocyte proportions, which can limit interpretation, and one of them used isoflurane for blood collection, which can influence leukocyte counts (Jacobsen et al. 2004).

As part of a larger study, we evaluated total leukocyte counts and differentials in free-ranging deermice sampled in 2016–18 near Charlo, Montana, US. We trapped deermice at four grids once a month, two grids at a time (Kuenzi et al. 2001). Two grids were trapped in October–November 2016, February–May 2017 (one of them again in June 2017), August–December 2017, and March–July 2018. The others were trapped in October–November 2017 and March–August 2018. We used Sherman traps (LNA model, H.B. Sherman, Tallahassee, Florida, USA) with peanut butter, oats, and polyester bedding. Traps were opened around dusk and checked about 4 h later. Captured deermice were ear tagged, sampled for feces, and returned to their traps until dawn, when they were weighed, sampled for feces and blood, and evaluated for sex, reproductive status, and fleas. Return of deermice to traps was necessary because, as part of the larger study, we needed to collect a second fecal sample for quantifying corticosterone metabolites after overnight confinement. Age was estimated from weight (nonadults ≤17 g, adults >17 g; Fairbairn 1977). Reproductive status was determined via presence of a perforate vagina, pregnancy or lactation in females, and scrotal testes in males. We collected blood from the retro-orbital capillary sinus using heparinized capillary tubes after the selected eye was anesthetized with proparacaine (Akorn, Inc., Lake Forest, Illinois, USA). Blood smears were briefly fixed in 100% methanol. We released individuals after processing and followed guidelines to prevent infection with Sin Nombre hantavirus (SNV; Mills et al. 1995).

Smears were stained with modified Wright's stain (Sigma-Aldrich, St. Louis, Missouri, USA) and were evaluated for total leukocyte counts and differentials using light microscopy. Leukocyte counts were estimated by counting leukocytes from the feathered edge toward the center for 20 fields at 400×. This provides a rough estimate of leukocyte counts, which allows for calculation of differential counts. The mean leukocyte count of 20 fields was calculated and multiplied by 2,000 to get a leukocyte count estimate (cells/μL) of blood. We identified and counted lymphocytes, neutrophils, monocytes, eosinophils, and basophils out of a sample of 100 leukocytes (whenever possible), counted at 1,000× (Woerpel and Rosskopf 1984). We calculated differential

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TABLE 1. Model statistics for linear mixed models with neutrophil, lymphocyte, or monocyte counts as the response variable from free-ranging deermice (*Peromyscus maniculatus*) captured in Montana, USA. All data are from adults (n=215), of which some were sampled in more than one season. Models were tested for significance using type III analysis of variance and Satterthwaite's method for estimating df. Significant results ($P \leq 0.05$) shown in bold. Season levels were fall–winter, spring, and summer.

Leukocyte	Variable	Sum of squares	Mean of squares	df	*F* value	*P* value
Lymphocytes	Sex	0.02	0.02	1/175.45	0.04	0.839
	Season	**14.05**	**7.02**	**2/185.32**	**15.91**	**<0.001**
	Reproductive status	0.44	0.44	1/193.36	0.99	0.321
Neutrophils	Sex	0.46	0.46	1/193.66	0.89	0.345
	Season	**3.87**	**1.94**	**2/210.28**	**3.74**	**0.025**
	Reproductive status	0.31	0.31	1/215.75	0.59	0.441
Monocytes	Sex	0.27	0.27	1/192.31	0.26	0.614
	Season	**13.72**	**6.86**	**2/217.83**	**6.58**	**0.002**
	Reproductive status	1.13	1.13	1/222.69	1.08	0.300

counts by multiplying proportions by total leukocyte counts.

We used linear mixed-effect models to examine whether season, sex, and reproductive status influenced lymphocyte, neutrophil, and monocyte counts. We did not do this for eosinophils and basophils because of low counts. Season was determined by grouping September–February into fall–winter, March–May into spring, and June–August into summer. Prior to analyses, we averaged leukocyte counts for deermice trapped more than once in the same season. We also natural log–transformed counts and added 10 points only to monocyte counts to improve normality and homoscedasticity of residuals. Deermouse identification was set as a random effect, because we sampled deermice (n=40) in more than one season. We did analyses in R (R Development Core Team 2018) within RStudio (RStudio Team 2015) using the lmerTest package (Kuznetsova et al. 2017) for linear mixed models. The initial model structure was leukocyte count as a function of sex×season×reproductive status. Because we had three leukocyte types, we ran three models and considered statistical significance at α=0.05. We used type III analysis of variance with Satterthwaite's method to test for significance. Because no interactions were significant, we reran the models with an additive structure. To generate reference tables, we used descriptive statistics.

We trapped 319 unique deermice, mostly adults (n=243), which were tested, as part of our larger study, for SNV antibodies using an enzyme-linked immunosorbent assay (Schountz et al. 2007). Therefore, we focused on adults that were SNV negative (i.e., adults with no antibodies), had no fleas (as determined in the field), and had leukocyte information available (n=215). Season was a significant predictor for neutrophil (F=3.74, P=0.025), lymphocyte (F=15.91, $P<0.001$), and monocyte (F=6.58, P=0.002) counts, but sex and reproductive status were not ($P \geq 0.300$; Table 1). Thus, we partitioned reference ranges by season (Table 2).

Our study generated leukocyte differential counts from blood collected using proparacaine in free-ranging deermice across seasons. Orozco (2015) did not test for seasonal differences, and McLean et al. (2012) reported only leukocyte proportions from summer months. Although Orozco (2015) found that males and females had similar neutrophil and lymphocyte proportions, they found that males had a higher monocyte proportion, in contrast to our findings. We acknowledge two caveats in our study. First, we handled deermice twice before collecting blood. However, because we processed them briefly at the first check, stress-related effects on leukocytes

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TABLE 2. Summary of leukocyte absolute counts for free-ranging deermice (*Peromyscus maniculatus*) from Montana, USA. All data are from adults (n=215), of which some were sampled in more than one season. Because only season influenced lymphocyte, neutrophil, and monocyte counts, data were accordingly partitioned.

Season	Leukocyte	No.	Cell counts		
			Mean (SD)	Median	Range
Fall–winter	Lymphocytes (10^3/μL)	73	1.77 (1.22)	1.52	0.17–5.85
	Neutrophils (10^3/μL)	73	1.16 (0.88)	1.00	0.08–3.85
	Monocytes (10^3/μL)	73	0.39 (0.40)	0.34	0.02–2.31
	Eosinophils (10^3/μL)	73	0.13 (0.30)	0.02	0.00–1.88
	Basophils (10^3/μL)	73	0.01 (0.02)	0.00	0.00–0.09
Spring	Lymphocytes (10^3/μL)	106	1.14 (0.97)	0.96	0.05–7.91
	Neutrophils (10^3/μL)	106	1.63 (1.26)	1.34	0.05–7.01
	Monocytes (10^3/μL)	106	0.49 (0.58)	0.31	0.00–2.98
	Eosinophils (10^3/μL)	106	0.10 (0.14)	0.04	0.00–0.65
	Basophils (10^3/μL)	106	0.01 (0.02)	0.00	0.00–0.09
Summer	Lymphocytes (10^3/μL)	67	0.80 (0.66)	0.50	0.03–2.63
	Neutrophils (10^3/μL)	67	1.35 (1.56)	0.97	0.08–8.73
	Monocytes (10^3/μL)	67	0.25 (0.48)	0.13	0.00–3.63
	Eosinophils (10^3/μL)	67	0.10 (0.25)	0.02	0.00–1.90
	Basophils (10^3/μL)	67	0.01 (0.03)	0.00	0.00–0.18

were most likely minimal. Second, because we did not trap all sites concurrently, temporal effects may have influenced leukocytes. Regardless, our reference ranges still provide a comprehensive view of leukocyte counts across seasons. Because leukocyte counts are influenced by the anatomical site of blood collection (Nemzek et al. 2001), we emphasize that our results will be most applicable for blood collected from the retro-orbital sinus.

The authors thank J. S. Knoll and P. Shaw for technical advice; S. Kirejczyk for her editorial comments; Montana Fish, Wildlife & Parks for land access; A. Kuenzi for traps; and numerous students for field and laboratory assistance. The authors acknowledge support from the University of Montana and the National Science Foundation under grant 1836793.

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Submitted for publication 23 April 2019.
Accepted 30 December 2019.

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