University of Nevada, Reno

Comparison of the Leukocyte Response to Infection with *Borrelia hermsii* in *Callospermophilus lateralis*, *Peromyscus maniculatus* and *Tamias amoenus*

A thesis submitted in partial fulfillment
of the requirements for the degree of

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by

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BACHELOR OF SCIENCE, VETERINARY SCIENCE

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Abstract

The purpose of this study was to establish baseline white blood cell (WBC) differential values in various non-infected rodent species and to determine whether infection by *Borrelia hermsii* affects their WBC composition. This research utilized blood samples collected from a variety of locations in the Sierra Nevada Mountains over several consecutive years. Each sample was tested for infection by *B. hermsii* using quantitative polymerase chain reaction. One hundred fifty blood smears from non-infected rodents (50 from each species) and 52 blood smears from *B. hermsii* infected rodents were chosen for the purpose of this study. Samples were stained using Wright-Giemsa stain and 100 white blood cells from each slide were visually located and identified using a light microscope. Establishing white blood cell differential parameters in non-infected rodents from these species will provide the scientific community with an expected range for their WBC compositions. Determining the immunologic response to infection by *B. hermsii* will give insight into the role of rodents as reservoirs in the ecology of tick-borne relapsing fever.

Acknowledgments

First and foremost, I would like to thank my thesis mentor, Dr. Mike Teglas, for agreeing to take on this project with me and helping me every step of the way and I would like to thank Dr. Tamara Valentine for her time and efforts towards providing the honors students with an invaluable thesis experience. Lastly, a big thank you to Jennifer Hsueh for helping me get set up in the lab and for answering all my questions so thoroughly. I appreciate all the help I received from my friends, family and UNR faculty towards completing my thesis project.

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Introduction

Description of tick-borne relapsing fever

Tick-borne relapsing fever (TBRF) is a zoonotic disease that results from infection by one of multiple species of bacteria belonging to the genus *Borrelia*. In humans, the disease is characterized by recurring periods of fever for several weeks duration, each period lasting anywhere from four to fourteen days with five to seven day intervals in between during which symptoms are not experienced (Forrester et al., 2015). The most common symptoms include abdominal pain, vomiting, and an altered sense of perception (Roscoe & Epperly, 2005). The reason TBRF manifests itself in the form of reoccurring fevers is due to a process known as antigenic variation. *Borrelia* spirochetes possess the ability to modify the proteins on their outer surface allowing them to temporarily avoid the host's immune response system until their numbers recover (Forrester et al., 2015). Humans become infected when bitten by a tick carrying TBRF-causing bacteria. In the United States, argasid ticks (soft ticks) belonging to the genus *Ornithodoros* serve as the vector for these pathogens (Oliver, Liles, & Spach, 1996). In North America, *Ornithodoros* ticks are most commonly infected with *Borrelia hermsii* and *Borrelia turicatae* bacteria but they may also be infected with *Borrelia parkeri* (Roscoe & Epperly, 2005). Tick-borne relapsing fever is found throughout most of the world. It is endemic (occurs frequently and at a predictable rate) in the western United States, southern British Columbia, the plateau regions of Mexico, Central and South America, the Mediterranean, Central Asia and throughout much of Africa (Dworkin, Schwan, Anderson, & Borchardt, 2008; Dugdale, 2014). In the U.S., TBRF has a mortality rate of up to 5% if left untreated (Van Dam, Van Gool, Wetsteyn, & Dankert, 1999). However, fatalities are rare in treated patients. Treatment of TBRF with a tetracycline or macrolilde antibiotic is typically effective (Roscoe & Epperly, 2005).

Transmission in the western United States

Cases of tick-borne relapsing fever have been reported in California, Montana, Texas, Washington, Arizona, Nevada, Idaho, Kansas, New Mexico, Utah, Oregon, Oklahoma and Colorado (Dworkin et al., 2008; Forrester et al., 2015). People afflicted by TBRF often report exposure in rustic cabins, summer vacation homes and permanent residences where wild rodents harboring these ticks have invaded the home (Goubau, 1984). In the western U.S., the most common cause of tick-borne relapsing fever is by infection with the spirochete bacteria *Borrelia hermsii* (Ryan & Ray, 2004). The endemic status of TBRF is maintained by a relationship between the tick vector *Ornithodoros hermsi*, *Borrelia hermsii* and animals that serve as hosts. *Borrelia hermsii* is typically transmitted by *O. hermsi* in forested areas ranging from <2,000 feet to >7,000 feet in elevation (Dworkin, Shoemaker, Fritz, Dowell, & Anderson, 2002). *Ornithodoros hermsi* feeds on small mammals, particularly rodents, with the most favored host being western chipmunks and squirrels (Wheeler, 1943). Rodents serve as primary reservoirs for *B. hermsii*; they harbor the bacteria in their bloodstream and may serve as a continuous source of infection. Ticks can become infected themselves by feeding on infected animals (Dworkin, Anderson, Schwan, Shoemaker, Banerjee, Kassen, & Burgdorfer, 1998). These ticks can transmit the disease to other animals, including humans, through their bites.

Introduction of species

In a study by Nieto and Teglas (2014) traps were placed in the Sierra Nevada and San Bernardino mountains in sites where human infection by *Borrelia hermsii* has historically or recently occurred. Among the rodents captured, chipmunks (*Tamias* spp.), deer mice (*P. maniculatus*) and golden-mantled ground squirrels (*C. lateralis*) were found to be most consistently infected with *B. hermsii*, suggesting that these animals have the potential to serve as

reservoirs for it in the western United States by maintaining it in nature. Thus, these rodents may be maintaining it in the locations where they were caught. In another study by Dworkin et al. (1998), 182 TBRF cases were compiled and analyzed in an attempt to create a detailed review of the disease. Forty-six of the cases identified the rodents that were present at the sites of exposure. Of these, 41.3% indicated the presence of mice, 34.8% indicated squirrels, 30.4% indicated chipmunks, and 13% indicated rats. Additionally, a study by Fritz, Payne and Schwan (2013) collected rodents from 23sites in California and tested them for antibodies to *B. hermsii* in an attempt to determine the primary hosts involved in maintaining *B. hermsii* in regions endemic for TBRF. Of the rodents tested, 36% of collected chipmunks (*Tamias* spp.), 21% of ground squirrels (*Callospermophilus lateralis* and *Otospermophilus beecheyi*) and 1.5% of *Peromyscus maniculatus* were antibody positive. The study suggested that *P. maniculatus* yielded a low percentage of antibody positive individuals because deer mice may have a more significant role in the ecology of TBRF in areas north of California. In order to expand the body of literature available on tick-borne relapsing fever and to understand how TBRF is maintained in nature, this study utilized *C. lateralis, P. maniculatus* and *T. amoenus* as model organisms to examine the relationship between *B. hermsii* and its rodent reservoirs.

Current study and hypotheses

The purpose of this study was to establish baseline parameters for white blood cell (WBC) differentials in small mammal hosts of tick-borne relapsing fever- *Callospermophilus lateralis* (golden-mantled ground squirrel), *Peromyscus maniculatus* (deer mouse) and *Tamias amoenus* (yellow-pine chipmunk) – and to determine the effects of sex and infection-status by *Borrelia hermsii* on the white blood cell composition of these rodents. Establishing white blood cell differential parameters in non-infected *C. lateralis, P. maniculatus* and *T. amoenus* provides

the scientific community with an expected range for each type of white blood cell (neutrophils, lymphocytes, monocytes, eosinophils and basophils) in these species. When values above or below these ranges are obtained from a white blood cell differential, health complications (such as infection) should be suspected in these animals. Determining the immunologic response to infection by *B. hermsii* in these rodent species gives insight into their role as host animals and reservoirs in the ecology of tick-borne relapsing fever. One hundred fifty blood smears from non-infected rodents (50 from each species) and 52 blood smears from *B. hermsii* infected rodents were chosen for the purpose of this study. Infection-status was tested using quantitative polymerase chain reaction (qPCR), an extremely sensitive and specific tool for detecting active infection with this pathogen (Mehra, Londoño, Sondey, Lawson, & Cadavid, 2009). Blood smears were made from each animal at the time of collection, and slides were stained in the laboratory using Wright-Giemsa stain in order to perform white blood cell differentials.

The null hypothesis that significant differences will not be observed in hematologic values within golden-mantled ground squirrels, deer mice and yellow-pine chipmunks when compared by sex and infection-status was tested. Previous studies have shown that neither sex nor infection by *Borrelia burgdorferi* (a close relative of *B. hermsii*) has an effect on the numbers of white blood cell types found in wild white-footed mice (*Peromyscus leucopus*)(Brisson, Ostfeld, Schwanz, & Voordouw, 2011; Wu, Greeley, Hansen, & Segre, 1999). The nearly absent hematologic response to infection by *Borrelia burgdorferi* (the pathogen that causes Lyme disease) in *P. leucopus* indicates that this rodent has the capacity to serve as a competent host for *B. burgdorferi* since it mounts a minimal immune response. Weak immune responses to bacterial infections due to an animal's inability to recognize the infection is a trend that is often seen in the relationships that develop between pathogens and their reservoir

hosts (Brunet, Sellitto, Spielman, & Telford III, 1995). If my hypothesis is correct the findings could mean that *C. lateralis, P. maniculatus* and *T. amoenus* also fail to adequately respond to the *Borrelia hermsii* pathogen and can thereby serve as consistent sources of the pathogen for naive tick vectors and potentially for human hosts bitten by infected ticks. Failure to respond may also represent a coevolution between the rodent hosts and *B. hermsii* bacteria, hinting at an evolution of virulence in these spirochetes in which they have become less harmful. (Alizon, 2008). If my hypothesis is not supported and an immune response is observed, the findings could suggest that these hosts show overt disease which may affect their role as reservoirs of the pathogen. Further studies involving experimentally infected and naturally infected wild rodents would be needed in order to confirm that infection by *Borrelia hermsii* compromises the health of rodent hosts.

White blood cell differentials

Rodents, like other mammals, have five types of white blood cells (leukocytes) which are split into two categories: granular (segmented neutrophils, eosinophils and basophils) and agranular (monocytes and lymphocytes) white blood cells (Jenkins, 2008). A white blood cell differential, also known as a leukocyte differential, is a diagnostic test that yields the percentage of each type of WBC present in an organism's blood. In addition to giving the percentages of the types of white blood cells mentioned above, WBC differentials reveal the percentage of abnormal or immature cells. For example, in this study the percentage of banded (immature) neutrophils was also recorded. Possessing leukocyte differential parameters for an organism is helpful in diagnosing the cause of an illness (bacterial vs. viral infections or, allergic and immune disorders) by comparing results to established "normal values" (labtestsonline.org). This study was aimed at expanding the literature available for these parameters in the wild small

mammal species: *Callospermophilus, Peromyscus* and *Tamias.* Once established, white blood cell differential parameters for these species can be used to determine how their immune system responds to various conditions (such as infection and immune disorders). For example, some infections trigger a response by the immune system that causes an increase in certain types of white blood cells, thereby altering the percentage of white blood cells they make up. An increase in the percent of neutrophils (a condition known as neutrophilia) could be indicative of acute bacterial infections, inflammation, tissue-death or smoking (labtestsonline.org). Whereas a decrease in neutrophil numbers (a condition known as neutropenia) could suggest a severe or chronic infection, drug use or an autoimmune disorder (labtestsonline.org). This study will determine whether or not the species in question mount a recognizable immune response to infection by *Borrelia hermsii* by observing differences in WBC percentages between the infected and non-infected populations.

Existing WBC differential parameters

Established hematology reference values for small mammals are difficult to obtain due to the variation that exists in blood collection techniques and laboratory procedures. This issue is further complicated when taking into account that the blood composition of small mammals can vary depending on multiple factors including age, gender, diet, reproductive state, hormones (Doeing, Borowicz, & Crockett, 2003) and season. Additionally, as small vertebrates are often prey animals, they can become easily stressed (due to handling, capture methods, or their environment), which also has an effect on their blood composition (Campbell & Ellis, 2007). With the aforementioned concerns in mind, few published references ranges are available for the white blood cell compositions of *Peromyscus maniculatus* (Blom, Gerber, & Nelson, 1994; McLean, Kelly, Molinar, Ghachu, Hart, O'Brien, Wright, Schountz, Hartney, & Lehmer, 2012)

and *Tamias amoenus* (Barker & Boonstra, 2005). Reference values are even rarer for *Callospermophilus lateralis.* However, there are some numbers available for species belonging to the closely related *Spermophilus* genus (Bouma, Strijkstra, Boerema, Deelman, Epema, Hut, Kroese, & Henning, 2010; Strauss, Mascher, Palme, & Millesi, 2007). This study will contribute to the eventual establishment of reliable reference ranges for leukocyte parameters in these species. For rodent WBC differentials, most studies report a composition of leukocytes in which neutrophils and lymphocytes make up the highest percentage followed by monocytes and eosinophils (Campbell & Ellis, 2007); basophils are typically the least common (see Table 1).

Table 1. White blood cell counts and differentials of hamsters (*Mesocricetus auratus)* and cotton rats (*Sigmodon hispidus*).

	Total WBC ($x10^3$ cells/µl)	**Lymphocytes (%)**	**Neutrophils (%)**	**Eosinophils (%)**	**Basophils (%)**	**Monocytes (%)**
Rabbit	5.2-12.5	30-85	20-75	0-5	0-10	0-10
Guinea pig	6-17	30-80	20-60	0-7	0-3	1-12
Hamster	3-15	50-96	17-35	0-5	0-5	0-5
Gerbil	4.3-21.6	32-97	2-41	0-4	0-2	0-9
Chinchilla	4-25	19-98	9-78	0-9	0-11	0-6
Deer mouse	1-4	N/A	N/A	N/A	N/A	N/A
Kangaroo rat	3-9	N/A	N/A	N/A	N/A	N/A
Cotton rat	11-13	50-54	36-40	5-6	0-0.2	4-5

Source: Mark A. Suckow, Karla A. Stevens, and Ronald P. Wilson, *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents* (San Diego: Elsevier Inc., 2012) 96. Print, table 3.10.

However, as can be seen in Table 1, actual leukocyte numbers may vary greatly. For example, hamsters (*Mesocricetus auratus*) may have up to 96% of their white blood cells comprised of

lymphocytes, whereas the cotton rat (*Sigmodon hispidus*) may only have up to 54% (Suckow, Stevens, & Wilson, 2012). In a study by Barker and Boonstra (2005), blood samples from yellow-pine chipmunks (*Tamias amoenus*) contained roughly 16.1-23.9% neutrophils, 66.4-76.3% lymphocytes, 2-3% monocytes, 1-2% eosinophils and 0-2% basophils. Considering that there are many factors that alter the results of a white blood cell differential, it will be interesting to see how the values I obtain on the same species compare to the values reported in the study by Barker and Boonstra (2005). The resulting differential parameters from this study coupled with existing ones can provide baseline hematologic values for future researchers who study the same species and wish to compare their values with existing ones.

Influence of infection-status and sex

Many published studies investigate the manner in which *Borrelia hermsii* is able to evade the immune system through antigenic variation of its outer surface proteins (Forrester et al., 2015; cdc.gov). However, studies rarely focus on the direct impact of *Borrelia hermsii* infection on white blood cell composition. This type of research is especially uncommon in wildlife, specifically *Callospermophilus lateralis, Peromyscus maniculatus* and *Tamias amoenus*. Knowing how the immune system responds to infection with *Borrelia hermsii* can be helpful in future epidemiologic studies and in understanding how diseases such as tick-borne relapsing fever (TBRF) are able to remain endemic in a given region. Of the studies which do exist on the composition of white blood cells, many focus on the fluctuation of their leukocyte numbers in response to hibernation and change in day length (Blom et al., 1994; Bouma et al., 2010). These types of studies add to the understanding of the effects of seasonality on white blood cell composition. A study on the difference in hematologic values between male and female adult sand rats (*Psammomys obesus*) found that sex is an important variable to take into consideration

when researching wild rodent populations (Kane, Steinbach, Sturdivant, & Bruks, 2012). The study found that in male sand rats a higher percentage of white blood cells were comprised of monocytes. Conversely, a study conducted on a strain of laboratory mice found no significant differences in white blood cell counts between males and females when blood was collected from the tail, foot and saphenous veins of the specimens. Interestingly, in this same study, a significant difference was found between male and female mice in the percentage of neutrophils when blood was collected via cardiac puncture (from the heart) (Doeing et al., 2003). Neutrophil numbers in female mice, as compared to males, were significantly lower in blood circulating through the heart. Researchers hypothesized that the difference in neutrophil numbers was attributable to a change brought about by female hormones such as estradiol and progesterone. It has been shown that female humans, when menstruating, also exhibit lower neutrophil numbers compared to males (Doeing et al., 2003). Thus, discoveries such as these can be applicable to humans and add to the understanding of human white blood cell fluctuations and hematology.

Materials and Methods

Collection and selection of samples

Callospermophilus lateralis, *Peromyscus maniculatus and Tamias amoenus* were previously captured, permanently identified with a radio-frequency tag and bled via abrasion of the retro-orbital sinus, as part of an ongoing research project at the University of Nevada, Reno (Nieto & Teglas, 2014). Captured rodents were anesthetized using isoflurane to make blood collection possible. Approximately 100 to 200 µl of blood was collected from each trapped animal based on its size. Blood was held in tubes containing an anticoagulant, ethylenediaminetetraacetic acid (EDTA), and placed on ice until transferred to the laboratory. Collected blood from all organisms was analyzed to determine if animals were infected with *Borrelia hermsii* using a genus specific TaqMan-PCR assay (Mehra et al., 2009) and the samples were also used to prepare blood smears resulting in an extensive collection of blood smears from both infected and non-infected *Callospermophilus lateralis*, *Peromyscus maniculatus* and *Tamias amoenus* rodents. Data collected on the captured rodents included: date of capture, location, longitude and latitude at site of capture, sex, weight and approximate age (adult vs. juvenile).

I selected two hundred and two samples, from a collection of nearly 2,000 blood samples, for use in this study. All samples chosen were collected between 2008 and 2011 during the months of April-November. Collection sites were located in California and Nevada and showed a history of human TBRF cases. Each animal's approximate age was determined by his or her weight at the time of collection and by the morphology of their genitalia. Only adults were chosen for this study to control for leukocyte variability associated with age. One hundred fifty samples that were negative for *Borrelia hermsii* infection, 50 from each species, were divided

evenly between males and females. These 150 blood smears made up the "non-infected population." After sorting through the samples from animals known to be infected with *B. hermsii* by molecular methods, the final "infected population" consisted of 52 blood smears from *C. lateralis* (*n*=8), *P. maniculatus* (*n*=17) and *T. amoenus* (*n*=27). Samples were omitted if their blood smears were not done properly, if their blood smears had been previously stained by methods unsuited for this study, or if only one blood smear was available for the specimen that it came from. Sample sizes for each species were too small to divide them evenly between males and females while retaining a large enough "infected population" for each.

Staining procedures

All slides were stained using a Wright-Giemsa hematology staining kit (volusol.com) for leukocyte differential cell analysis. Standard staining techniques used for identification of bacteria were not appropriate in a study of this type. Special staining techniques (outlined below) were required for this project in order to accurately identify cellular features of each leukocyte type, in both the nucleus and cytoplasm, allowing for accurate identification of the individual cell type. Several staining techniques were tested initially, such as Romanowsky, Diff-Quick and Wright-Giemsa stains, and a technique was found using a Wright-Giemsa stain that allowed for accurate identification of rodent white blood cell types. Four containers with tightly fitting screw caps were labeled 1-4 and filled with 1) methanol, 2) Volu-Sol's Wright-Giemsa stain, 3) Volu-Sol's Hematology Buffer and 4) Volu-Sol's Hematology Rinse, respectively. Staining procedures were as follows. First, blood smears were fixed (adhered to the slide) by dipping them in methanol for approximately 5 minutes. The slide was then dipped in the stain for approximately 60 seconds; care was taken not to agitate the slide. Afterwards, the slide's edge was blotted to remove excess stain. The same procedure was repeated with each slide using

buffer (approximately 60 seconds). Lastly, the slide was dipped in the rinse solution for approximately 10 seconds and then the edge was blotted. Slides were allowed to completely dry in a vertical position on an absorbent surface before microscopic examination. The buffer was changed when a scum of stain became noticeable on its surface or when the buffer became discolored to a dark blue. Rinse was changed when it became a medium blue. Stain was replaced when its volume became insufficient (volusol.com).

Leukocyte differentials

Two white blood cell differentials were performed on all selected slides. The second WBC differential was done to improve the accuracy of the counts. Differential leukocyte counts were performed on all blood smears in the following manner: one hundred leukocyte cells for each slide were identified and the proportions of each white blood cell type (segmented neutrophils, lymphocytes, monocytes, eosinophils and basophils) and banded neutrophils were expressed as percentages. Leukocyte differentials were performed manually by following a systematic scanning path. First, the thinnest edge of the blood smear (which ideally will look like the edge of a feather or "finger-like") was located. Using the 40x objective lens of a light microscope, cells were counted and identified by scanning across the slide from one side to another within the lens' field of view and then adjusting the slide to an adjacent field without overlapping the previous one so as to prevent recounting individual cells (cshlpress.com). This process was continued until 100 cells were counted.

Statistical analyses

Two white blood cell differentials were performed on all slides, thus, averages were calculated for the number of segmented neutrophils, lymphocytes, monocytes, basophils, eosinophils and banded neutrophils found for each individual. Using these numbers, averages

and the standard deviation (SD) of the percentages for each type of white blood cell were obtained for each species (infected and non-infected populations) and each sex. All data were expressed as means ± standard deviation. Statistical analysis was performed to determine if there were any significant differences between sex and infection-status. Comparisons between sex and infection-status within each species were done using an unpaired student's *t*-test analysis. Values of $p \leq 0.05$ were considered significant. *Minitab version 17 software*© was used to complete statistical calculations.

Results

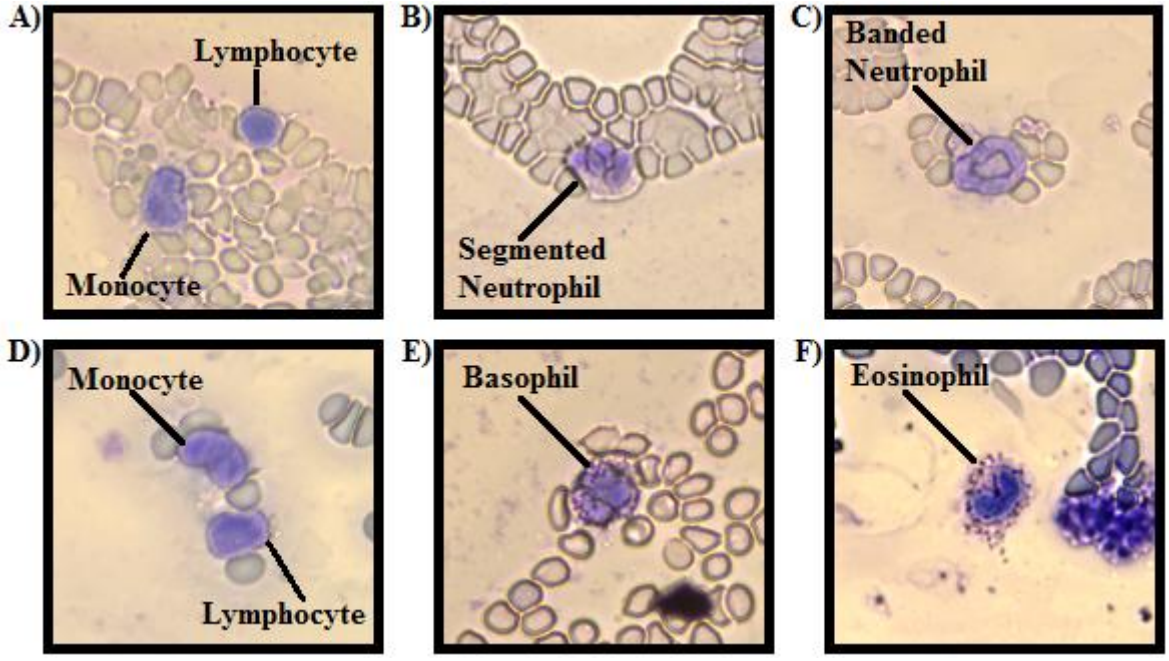
Sample size

One hundred fifty samples, 50 from each species, were utilized to represent the "non-infected populations" for *Callospermophilus lateralis*, *Peromyscus maniculatus* and *Tamias amoenus*; samples were split evenly between males and females. Fifty-two total samples were included in the *Borrelia hermsii* infected population: eight samples from *C. lateralis* specimens, 17 from *P. maniculatus* and 27 from *T. amoenus*. Sample size numbers in the "infected populations" were insufficient to make statistical comparisons between sexes within the species, especially in the case of *C. lateralis* (5 females, 3 males).

Blood smear observations

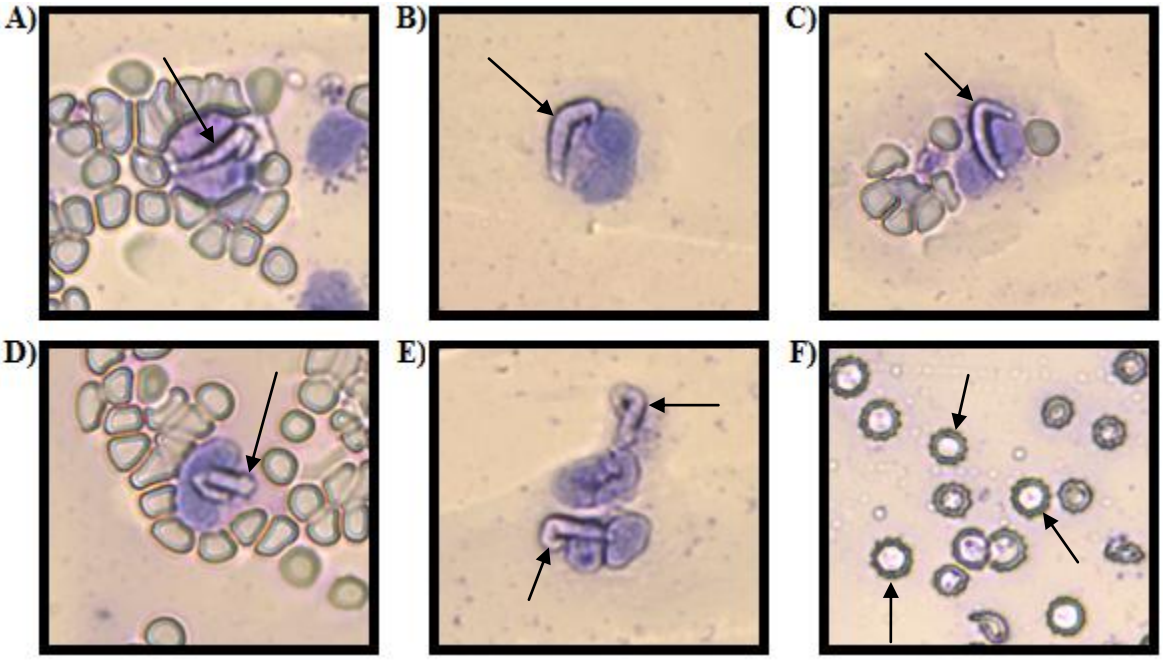
The use of Wright-Giemsa stain for this study proved effective in preparing blood smears for white blood cell (WBC) differential analysis. Wright-Giemsa stain allowed for the visualization of multi-lobed nuclei, cytoplasm, granules and other features key to identifying each white blood cell type (see Figure 1). Additionally, the use of Wright-Giemsa stain allowed

Figure 1. Images of 6 white blood cell types viewed under 40x magnification. Images captured from the following specimens: A, C, F) *Peromyscus maniculatus* B) *Tamias amoenus* D, E) *Callospermophilus lateralis*



for the ability to distinguish between the reddish-purple and dark purple granules of eosinophils and basophils, respectively. Differences in the cell morphology between species were not apparent for any white blood cell type. Figure 1 shows an example of a monocyte and a lymphocyte from the blood smear of a deer mouse (*Peromyscus maniculatus*)(top left image) and the same white blood cell types can be seen from the blood smear of a golden-mantled squirrel (*Callospermophilus lateralis*)(bottom left image). The monocytes and lymphocytes have similar shapes and appear to have a similar chromatin density in the nucleus as can be seen from the shade of purple that they stain. Although both cells appear slightly larger in the image from *C. lateralis*, this difference in size is due to variation within lymphocytes and monocytes rather than differences between the two species. A peculiar structure was found on some white blood cells solely from *T. amoenus* smears (see Figure 2). The occurrence of this structure did not seem to

Figure 2. Images of abnormalities found in blood smears viewed under 40x magnification. A-E) captured from various *Tamias amoenus* blood smears. F) Echinocytes found in a blood smear belonging to *Tamias amoenus* specimen.



be associated with any specific white blood cell type. Presence of this characteristic could be

found in most *T. amoenus* blood smears regardless of *B. hermsii* infection-status although, its presence seemed to decrease dramatically in the blood smears of infected *T. amoenus* specimens. Moreover, poikilocytosis (presence of abnormalities in the shape of blood components) was seen in the red blood cell structure of two *T. amoenus* specimens in which red blood cells appeared to have a spiked rather than a smooth outer surface. These spiculated red blood cells are termed echinocytes and may be a result of renal disease, lymphoma, exposure to certain drugs, or red blood cell dehydration in other animal species (Campbell & Ellis, 2007). An image of echinocytes was captured from a *Tamias amoenus* blood smear and can be seen in Figure 2F. Often, projections on the surface of red blood cells are associated with errors in blood smear preparation (e.g. blood films that are allowed to dry too slowly or because of excessive concentrations of EDTA) (Campbell & Ellis, 2007). In both cases where echinocytes were found, neither specimen's white blood cell differentials resulted in abnormal counts.

Leukocyte differentials

Observations made in this study were consistent with those stated in published studies (cshlpress.com). It is best to perform WBC differentials near the "fringed" or "finger-like" edge of the blood smear. In this area, the blood smear is least dense (if done appropriately) and allows for the identification of individual white blood cells. Components of the blood become increasingly dense and compact as it gets further from this fringed end, making it difficult to perform cell differentials. Moreover, white blood cells at the very edge of blood smears should not be counted in differentials. In these areas, the percentage of white blood cell types in a blood smear are not representative of the true composition, and some white blood cell type percentages may be skewed. This edge may contain an accumulation of segmented neutrophils, lymphocytes, eosinophils, or banded neutrophils. In some smears, a differential including cells at the very edge

of the smear resulted in significantly higher or lower numbers in any of the aforementioned white blood cell types compared to a differential excluding these regions. Thus, the optimal region for cell differential analysis is the area that lies between the edges of the fringed end and the thicker portion of the blood smear. When performing white blood cell differentials visually it is suggested to wait a period of time before carrying out the second differential in the same area in order to avoid performing a biased differential. However, if the optimal region for cell differential analysis is large enough one can count and identify 100 leukocyte cells and continue scanning the smear for the second differential as long as it remains uniform in appearance.

White blood cell reference ranges

Figures 3-5 (in appendix) display results from 150 total white blood cell differentials for non-infected *Callospermophilus lateralis, Peromyscus maniculatus* and *Tamias amoenus,* respectively. The charts display the number of segmented neutrophils (s.n.), lymphocytes, monocytes, basophils, eosinophils and banded neutrophils (b.n.) found in the first 100 white blood cells of every specimen for two different differentials. Blue columns contain the average numbers found for each white blood cell type calculated from the results of the two differentials. These numbers also represent the percentages of each WBC type found in each specimen; because counts were out of one hundred, no further calculations were needed to determine percentages. Figures 6-8 (in appendix) display the same information from the results of 52 total white blood cell differentials for *Borrelia hermsii* infected *C. lateralis, P. maniculatus and T. amoenus* respectively.

White blood cell differential results for all specimens for each species (in both infected and non-infected populations) were averaged and expressed as mean ± standard deviation. White blood cell reference values for non-infected *C. lateralis* (s.n.: 42.03 ± 16.82, lymphoctyes: 48.13

± 15.02, monocytes: 8.34 ± 3.62, eosinophils: 0.72 ± 1.27, basophils: 0.33 ± 0.63, b.n.: 0.45 ± 0.74), *P. maniculatus* (s.n.: 32.61 ± 12.68, lymphoctyes: 53.65 ± 12.10, monocytes: 9.53 ± 3.66, eosinophils: 2.5 ± 2.12, basophils: 0.66 ± 0.82, b.n.: 1.05 ± 1.12) and *T. amoenus* (s.n.: 35.31 ± 14.11, lymphoctyes: 55.93 ± 13.09, monocytes: 7.4 ± 3.18, eosinophils: 0.39 ± 0.61, basophils: 0.25 ± 0.47, b.n.: 0.72 ± 0.78) are summarized in Table 2 in the form of percentages.

Table 2. White blood cell differential values in *Borrelia hermsii* infected and non-infected *Callospermophilus lateralis, Peromyscus maniculatus* and *Tamias amoenus*. Sample size for each species represented by *n*. Values that differed significantly are highlighted in bold.

Species	*C. lateralis*		*P. maniculatus*		*T. amoenus*	
WBC type	Non-infected (n = 50)	Infected (n = 8)	Non-infected (n = 50)	Infected (n = 17)	Non-infected (n = 50)	Infected (n = 27)
% Segmented Neutrophil	42.03 ± 16.82	38.81 ± 10.82	32.61 ± 12.68	29.5 ± 13.50	35.31 ± 14.11	30.78 ± 12.67
% Lymphocyte	48.13 ± 15.02	50.06 ± 11.90	**53.65 ± 12.10**	**62.53 ± 14.40**	55.93 ± 13.09	61.69 ± 11.70
% Monocyte	8.34 ± 3.62	8.75 ± 2.98	**9.53 ± 3.66**	**3.97 ± 2.08**	7.4 ± 3.18	6.98 ± 3.12
% Eosinophil	0.72 ± 1.27	1.5 ± 1.10	2.5 ± 2.12	1.62 ± 1.64	0.39 ± 0.61	0.20 ± 0.44
% Basophil	0.33 ± 0.63	0.25 ± 0.53	0.66 ± 0.82	0.74 ± 0.81	0.25 ± 0.47	0.11 ± 0.29
% Banded Neutrophil	0.45 ± 0.74	0.63 ± 0.64	1.05 ± 1.12	1.65 ± 1.81	**0.72 ± 0.78**	**0.24 ± 0.38**

White blood cell reference values for *Borrelia hermsii* infected *C. lateralis* (s.n.: 38.81 ± 10.82, lymphoctyes: 50.06 ± 11.90, monocytes: 8.75 ± 2.98, eosinophils: 1.5 ± 1.10, basophils: 0.25 ± 0.53, b.n.: 0.63 ± 0.64), *P. maniculatus* (s.n.: 29.5 ± 13.50, lymphoctyes: 62.53 ± 14.40, monocytes: 3.97 ± 2.08, eosinophils: 1.62 ± 1.64, basophils: 0.74 ± 0.81, b.n.: 1.65 ± 1.81) and *T. amoenus* (s.n.: 30.78 ± 12.67, lymphoctyes: 61.69 ± 11.70, monocytes: 6.98 ± 3.12, eosinophils: 0.20 ± 0.44, basophils: 0.11 ± 0.29, b.n.: 0.24 ± 0.38) can also be seen in Table 2 in the form of percentages.

Additionally, for individuals in the "non-infected population" white blood cell differential results for each sex within each species were averaged and expressed as mean ± standard deviation. White blood cell reference values for non-infected *C. lateralis* males (s.n.: 39.98 ± 18.44, lymphoctyes: 49.7 ± 16.34, monocytes: 8.34 ± 3.72, eosinophils: 0.96 ± 1.59, basophils: 0.44 ± 0.78, b.n.: 0.58 ± 0.86) and females (s.n.: 44.08 ± 15.12, lymphoctyes: 46.56 ± 13.72, monocytes: 8.34 ± 3.60, eosinophils: 0.48 ± 0.80, basophils: 0.22 ± 0.41, b.n.: 0.58 ± 0.86) are summarized in Table 3 in the form of percentages.

Table 3. White blood cell differential values in male and female *Callospermophilus lateralis*, *Peromyscus maniculatus* and *Tamias amoenus*. Sample size for each species represented by *n*. Values that differed significantly are highlighted in bold.

Species	*C. lateralis*		*P. maniculatus*		*T. amoenus*	
WBC type	Male	Female	Male	Female	Male	Female
% Segmented Neutrophil	39.98 ± 18.44	44.08 ± 15.12	32.68 ± 13.07	32.54 ± 12.54	35.88 ± 15.99	34.74 ± 12.25
% Lymphocyte	49.7 ± 16.34	46.56 ± 13.72	51.9 ± 12.61	55.4 ± 11.56	55.16 ± 15.32	56.7 ± 10.68
% Monocyte	8.34 ± 3.72	8.34 ± 3.60	**10.64 ± 3.63**	**8.42 ± 3.40**	7.38 ± 2.77	7.42 ± 3.59
% Eosinophil	0.96 ± 1.59	0.48 ± 0.80	2.92 ± 2.15	2.08 ± 2.03	**0.56 ± 0.73**	**0.22 ± 0.41**
% Basophil	0.44 ± 0.78	0.22 ± 0.41	0.74 ± 0.93	0.58 ± 0.72	0.24 ± 0.41	0.26 ± 0.52
% Banded Neutrophil	0.58 ± 0.86	0.32 ± 0.59	1.12 ± 1.28	0.98 ± 0.96	0.78 ± 0.93	0.66 ± 0.61

White blood cell reference values for non-infected *P. maniculatus* males (s.n.: 32.68 ± 13.07, lymphoctyes: 51.9 ± 12.61, monocytes: 10.64 ± 3.63, eosinophils: 2.92 ± 2.15, basophils: 0.74 ± 0.93, b.n.: 1.12 ± 1.28) and females (s.n.: 32.54 ± 12.54, lymphoctyes: 55.4 ± 11.56, monocytes: 8.42 ± 3.40, eosinophils: 2.08 ± 2.03, basophils: 0.58 ± 0.72, b.n.: 0.98 ± 0.96) are summarized in Table 3 in the form of percentages.

White blood cell reference values for non-infected *T. amoenus* males (s.n.: 35.88 ± 15.99,

lymphoctyes: 55.16 ± 15.32, monocytes: 7.38 ± 2.77, eosinophils: 0.56 ± 0.73, basophils: 0.24 ± 0.41, b.n.: 0.78 ± 0.93) and females (s.n.: 34.74 ± 12.25, lymphoctyes: 56.7 ± 10.68, monocytes: 7.42 ± 3.59, eosinophils: 0.22 ± 0.41, basophils: 0.26 ± 0.52, b.n.: 0.66 ± 0.61) are summarized in Table 3 in the form of percentages.

Statistical analyses

Callospermophilus lateralis, Peromyscus maniculatus and *Tamias amoenus* were compared by sex using an unpaired *t*-test with a 95% confidence interval ($p \leq 0.05$) (see Table 3). Two groups exhibited significant differences. Monocytes were significantly lower in female *P. maniculatus* (8.42 ± 3.40) than in male mice (10.64 ± 3.63) with a P-value of 0.030 and eosinophils were significantly lower in female *T. amoenus* (0.22 ± 0.41) than in male mice (0.56 ± 0.73) with a P-value of 0.049. The values of all other male vs. female comparisons were not significant.

Statistical comparisons of infected and non-infected populations by species yielded three significantly different results. In *P. maniculatus,* lymphocytes were significantly higher in *B. hermsii* infected hosts (62.53 ± 14.40) than in non-infected specimens (53.65 ± 12.10) with a P-value of 0.032, whereas monocytes decreased from 9.53±3.66 % to 3.97±2.08 % in infected *P. maniculatus* hosts. For the statistical comparison between monocyte percentages in infected vs. non-infected *P. maniculatus, Minitab version 17 software*© reported a P-value of 0.000. This value may have been truncated or rounded by the software resulting in this erroneous P-value. Thus, the P-value for this comparison is less than 0.0001. Lastly, banded neutrophil percentages were significantly lower in *T. amoenus* infected with *B. hermsii*, dropping from 0.72±0.78 % (range: 0-3%) to 0.24±0.38 % (range: 0-1%) (P-value = 0.001).

Discussion

This paper establishes white blood cell reference ranges for segmented neutrophils, lymphocytes, monocytes, eosinophils, basophils and banded neutrophils in three wild rodent species (*Callospermophilus lateralis, Peromyscus maniculatus and Tamias amoenus*) which may serve as primary reservoir hosts for *Borrelia hermsii* and maintain their populations in areas endemic for tick-borne relapsing fever. White blood cell differential parameters are scarce in the scientific literature for these species. The methods consisted of staining 150 blood smears, prepared from previously captured wild rodents, with Wright-Giemsa stain and performing two manual white blood cell differential counts on each one. Blood smears consisted of blood from 50 wild rodents of each species (*C. lateralis, P. maniculatus* and *T. amoenus*) which tested negative for *Borrelia hermsii* infection using quantitative polymerase chain reaction. This paper also investigates the immune response of these three species to infection by *B. hermsii* and the effects of sex on their white blood cell compositions. Thus, 52 total blood smears from *C. lateralis* (*n*=8), *P. maniculatus* (*n*=17) and *T. amoenus* (*n*=27), which tested positive for *B. hermsii* infection, were also subjected to the aforementioned procedures.

Due to the high variation in leukocyte differential parameters, even amongst closely related rodent species (Suckow et al., 2012), it is difficult to determine the accuracy of a differential's results unless established values exist for the species of interest. Moreover, when reference ranges are available for a desired species, values may vary greatly from one study to another depending on factors such as the time of year the blood samples were drawn, how specimens were handled, methods of blood draw used and accuracy of differentials (Lang). Thus, assessing the validity of personally acquired differential results with published ranges should be done with caution. Consistent with previous studies (Campbell & Ellis, 2007), neutrophil and

lymphocyte percentages were highest in *C. lateralis, P. maniculatus* and *T. amoenus* followed by monocytes and eosinophils (see Table 3). Additionally, basophils and banded neutrophils were rare and often made up 0% of white blood cells in individuals belonging to all three species (see Figures 3-5). A study by Barker and Boonstra (2005) found the following ranges for leukocyte differentials in *Tamias amoenus*: 16.1-23.9% neutrophils, 66.4-76.3% lymphocytes, 2-3% monocytes, 1-2% eosinophils and 0-2% basophils. Results for *T. amoenus* leukocyte differentials (see Table 2) agreed with the white blood cell type ranges obtained from Barker and Boonstra's study for all cell types except monocytes. Monocyte numbers in non-infected *Tamias amoenus* ranged from 4.22-10.58%. This was higher than the monocyte range published in Barker and Boonstra's (2005) study (2-3%). This difference may be attributable to variability in blood collection methods (cardiac puncture versus abrasion of the retro-orbital sinus). Age can be ruled out as a source of variation as Barker and Boonstra's study also removed juveniles from their samples. The establishment of "expected" reference values for leukocyte differentials in *C. lateralis, P. maniculatus and T. amoenus* is useful for scientists who include these specimens in their studies and wish to compare the values that they obtain to existing ones.

Sixteen of the eighteen statistical comparisons carried out between males and females showed no significant differences in white blood cell numbers, consistent with my null hypothesis. The exceptions were higher monocyte numbers in male vs. female *P. maniculatus* and higher eosinophil numbers in male vs. female *T. amoenus*. Similar to my results, a study by Kane et al. (2012) found higher monocyte numbers in male sand rats (*Psammomys obesus*) compared to females. They note that sex-associated differences in percentage of monocytes may change with time and can even reverse at certain ages. A study by Doeing et al. (2003) stated that differences in white blood cell composition between sexes may arise from differences in

hormones between males and females.

The null hypothesis that significant differences would not be observed in hematologic values within species between non-infected and *Borrelia hermsii* infected wild rodents was supported in the majority of the statistical analyses. Lymphocyte percentages increased in infected *P. maniculatus*, monocyte percentages decreased in infected *P. maniculatus* and banded neutrophil percentages decreased in infected *T. amoenus* rodents. There were no significant differences in all the other white blood cell types. This indicates that a very minimal or nearly absent immune response was mounted against infection by *B. hermsii* in *T. amoenus* and a response was completely absent in *C. lateralis*. The significant differences in both monocytes and lymphocytes for *P. maniculatus* suggest that it may be a less competent reservoir host for *B. hermsii*. To determine if the health of *P. maniculatus* is actually physically compromised by *B. hermsii* infection further research would be needed. A study that monitors the behavioral and physical changes of infected versus non-infected *P. maniculatus* would be ideal in determining if an actual illness develops along with observing clinical signs.

Increased awareness of tick-borne relapsing fever and knowledge of the areas in which it is endemic could assist health professionals in accurately diagnosing TBRF cases in patients allowing them to administer proper treatment and thereby, decrease morbidity and mortality rates associated with this disease (Oliver et al., 1996). Properly identifying the primary host reservoirs of *Borrelia hermsii* could be helpful in decreasing the number of yearly cases in the western United States by providing information needed to create disease maps. Upon identification of these species, travelers and home-owners of cabins in rural or forested areas can be notified whether or not they are at risk for TBRF and if caution should be taken to prevent it if these species reside in the area. Preventative measures to ward off ticks while traveling can include the

use of insect repellents (containing DEET or permethrin), protective clothing and sleeping on elevated surfaces (Roscoe & Epperly, 2005). Other methods of prevention include: rodent-proof buildings in endemic regions, contacting a licensed pest control specialist to remove rodent nesting material from homes and pesticide treatments (cdc.gov).

Appendix

Figure 3. White blood cell differential reference ranges of 25 female (F) and 25 male (M) non-infected *Callospermophilus lateralis* (c.l.) specimens including segmented neutrophil (S.N.), lymphocyte, monocyte, basophil, eosinophil and banded neutrophil (B.N.) percentages. Two differentials were performed for each individual and results for each count were averaged (avg).

#	Species	Sex	S.N.	2	avg	Lymphocytes	2	avg	Monocytes	2	avg	Basophils	2	avg	Eosinophils	2	avg	B.N.	2	avg
F1	c.l.	F	48	48	48	44	45	44.5	7	6	6.5	0	0	0	1	1	1	0	0	0
F2	c.l.	F	85	88	86.5	12	9	10.5	2	3	2.5	1	0	0.5	0	0	0	0	0	0
F3	c.l.	F	52	54	53	35	33	34	12	11	11.5	0	0	0	1	2	1.5	0	0	0
F4	c.l.	F	39	41	40	50	49	49.5	9	9	9	1	0	0.5	1	1	1	0	0	0
F5	c.l.	F	39	44	41.5	55	51	53	6	5	5.5	0	0	0	0	0	0	0	0	0
F6	c.l.	F	60	61	60.5	35	33	34	5	6	5.5	0	0	0	0	0	0	0	0	0
F7	c.l.	F	40	43	41.5	44	40	42	10	11	10.5	1	1	1	3	3	3	2	2	2
F8	c.l.	F	17	18	17.5	77	74	75.5	6	8	7	0	0	0	0	0	0	0	0	0
F9	c.l.	F	63	63	63	31	33	32	6	4	5	0	0	0	0	0	0	0	0	0
F10	c.l.	F	34	31	32.5	56	58	57	10	11	10.5	0	0	0	0	0	0	0	0	0
F11	c.l.	F	32	33	32.5	58	57	57.5	9	10	9.5	1	0	0.5	0	0	0	0	0	0
F12	c.l.	F	35	33	34	56	61	58.5	8	5	6.5	0	0	0	0	0	0	1	1	1
F13	c.l.	F	30	31	30.5	54	56	55	14	11	12.5	0	0	0	0	0	0	2	2	2
F14	c.l.	F	14	12	13	74	72	73	10	14	12	0	0	0	1	2	1.5	1	0	0.5
F15	c.l.	F	44	43	43.5	41	44	42.5	14	12	13	1	1	1	0	0	0	0	0	0
F16	c.l.	F	58	58	58	33	36	34.5	5	4	4.5	2	1	1.5	1	1	1	1	0	0.5
F17	c.l.	F	27	29	28	64	61	62.5	8	10	9	0	0	0	1	0	0.5	0	0	0
F18	c.l.	F	38	42	40	45	39	42	15	16	15.5	0	1	0.5	2	2	2	0	0	0
F19	c.l.	F	54	51	52.5	34	36	35	12	13	12.5	0	0	0	0	0	0	0	0	0
F20	c.l.	F	46	42	44	50	54	52	4	4	4	0	0	0	0	0	0	0	0	0
F21	c.l.	F	50	53	51.5	43	42	42.5	6	4	5	0	0	0	0	0	0	1	1	1
F22	c.l.	F	43	40	41.5	43	46	44.5	13	14	13.5	0	0	0	1	0	0.5	0	0	0
F23	c.l.	F	54	51	52.5	41	46	43.5	4	3	3.5	0	0	0	0	0	0	1	0	0.5
F24	c.l.	F	49	48	48.5	46	43	44.5	5	8	6.5	0	0	0	0	0	0	0	1	0.5
F25	c.l.	F	49	47	48	43	46	44.5	8	7	7.5	0	0	0	0	0	0	0	0	0
M1	c.l.	M	30	31	30.5	50	50	50	12	11	11.5	1	0	0.5	7	8	7.5	0	0	0
M2	c.l.	M	39	42	40.5	46	44	45	10	8	9	3	2	2.5	1	3	2	1	1	1
M3	c.l.	M	14	13	13.5	74	78	76	9	8	8.5	0	0	0	3	1	2	0	0	0
M4	c.l.	M	51	54	52.5	40	36	38	7	6	6.5	1	1	1	1	2	1.5	0	1	0.5
M5	c.l.	M	51	52	51.5	38	34	36	7	9	8	0	0	0	2	4	3	2	1	1.5
M6	c.l.	M	57	62	59.5	36	32	34	7	6	6.5	0	0	0	0	0	0	0	0	0
M7	c.l.	M	48	45	46.5	45	47	46	7	8	7.5	0	0	0	0	0	0	0	0	0
M8	c.l.	M	13	15	14	80	81	80.5	5	3	4	2	1	1.5	0	0	0	0	0	0
M9	c.l.	M	27	27	27	61	63	62	12	10	11	0	0	0	0	0	0	0	0	0
M10	c.l.	M	39	37	38	53	57	55	8	6	7	0	0	0	0	0	0	0	0	0
M11	c.l.	M	23	22	22.5	57	61	59	17	13	15	0	0	0	1	0	0.5	2	4	3
M12	c.l.	M	42	38	40	40	42	41	16	17	16.5	0	0	0	0	1	0.5	2	2	2
M13	c.l.	M	32	33	32.5	58	59	58.5	8	8	8	0	0	0	1	0	0.5	1	0	0.5
M14	c.l.	M	54	58	56	42	36	39	4	5	4.5	0	0	0	0	0	0	0	1	0.5
M15	c.l.	M	54	50	52	39	42	40.5	7	7	7	0	0	0	0	0	0	0	1	0.5
M16	c.l.	M	23	20	21.5	60	65	62.5	12	11	11.5	1	2	1.5	1	0	0.5	3	2	2.5
M17	c.l.	M	20	20	20	64	62	63	15	17	16	0	0	0	1	1	1	0	0	0
M18	c.l.	M	68	70	69	23	22	22.5	9	8	8.5	0	0	0	0	0	0	0	0	0
M19	c.l.	M	62	60	61	31	33	32	6	6	6	0	0	0	1	1	1	0	0	0
M20	c.l.	M	55	56	55.5	38	39	38.5	6	5	5.5	0	0	0	1	0	0.5	0	0	0
M21	c.l.	M	21	18	19.5	71	73	72	6	8	7	0	0	0	2	1	1.5	0	0	0
M22	c.l.	M	14	13	13.5	74	71	72.5	9	13	11	1	1	1	1	0	0.5	1	2	1.5
M23	c.l.	M	75	74	74.5	25	23	24	0	2	1	0	0	0	0	0	0	0	1	0.5
M24	c.l.	M	30	29	29.5	59	62	60.5	7	5	6	2	3	2.5	2	1	1.5	0	0	0
M25	c.l.	M	58	60	59	36	33	34.5	5	6	5.5	1	0	0.5	0	0	0	0	1	0.5

Figure 4. White blood cell differential reference ranges of 25 female (F) and 25 male (M) non-infected *Peromyscus maniculatus* (p.m.) specimens including segmented neutrophil (S.N.), lymphocyte, monocyte, basophil, eosinophil and banded neutrophil (B.N.) percentages. Two differentials were performed for each individual and results for each count were averaged (avg).

#	Species	Sex	S.N.	2	avg	Lymphocytes	2	avg	Monocytes	2	avg	Basophils	2	avg	Eosinophils	2	avg	B.N.	2	avg
F1	p.m.	F	30	32	31	58	56	57	9	8	8.5	1	0	0.5	2	4	3	0	0	0
F2	p.m.	F	32	38	35	52	48	50	13	8	10.5	1	1	1	1	3	2	1	2	1.5
F3	p.m.	F	57	50	53.5	38	39	38.5	3	7	5	1	0	0.5	0	3	1.5	1	1	1
F4	p.m.	F	28	20	24	53	55	54	14	16	15	1	0	0.5	3	7	5	1	2	1.5
F5	p.m.	F	21	16	18.5	65	68	66.5	11	15	13	1	1	1	2	0	1	0	0	0
F6	p.m.	F	51	53	52	40	35	37.5	4	5	4.5	0	0	0	5	4	4.5	0	3	1.5
F7	p.m.	F	29	24	26.5	56	66	61	12	10	11	1	0	0.5	1	0	0.5	1	0	0.5
F8	p.m.	F	35	27	31	48	56	52	8	8	8	1	0	0.5	8	9	8.5	0	0	0
F9	p.m.	F	40	37	38.5	47	46	46.5	10	13	11.5	1	0	0.5	2	3	2.5	0	1	0.5
F10	p.m.	F	26	30	28	59	55	57	13	12	12.5	0	0	0	0	1	0.5	2	2	2
F11	p.m.	F	11	13	12	74	71	72.5	8	8	8	2	3	2.5	4	5	4.5	1	0	0.5
F12	p.m.	F	29	25	27	55	59	57	13	13	13	0	0	0	2	2	2	1	1	1
F13	p.m.	F	31	25	28	46	59	52.5	11	10	10.5	1	1	1	6	2	4	5	3	4
F14	p.m.	F	45	50	47.5	40	42	41	9	4	6.5	0	1	0.5	4	2	3	2	1	1.5
F15	p.m.	F	28	31	29.5	57	52	54.5	10	12	11	1	0	0.5	3	3	3	1	2	1.5
F16	p.m.	F	30	34	32	62	59	60.5	7	7	7	0	0	0	0	0	0	1	0	0.5
F17	p.m.	F	22	26	24	70	68	69	7	5	6	0	0	0	1	1	1	0	0	0
F18	p.m.	F	15	18	16.5	77	75	76	4	5	4.5	2	1	1.5	1	1	1	1	0	0.5
F19	p.m.	F	54	54	54	44	44	44	2	1	1.5	0	0	0	0	0	0	0	1	0.5
F20	p.m.	F	18	17	17.5	73	76	74.5	9	7	8	0	0	0	0	0	0	0	0	0
F21	p.m.	F	28	30	29	61	59	60	9	10	9.5	0	0	0	1	0	0.5	1	1	1
F22	p.m.	F	55	52	53.5	34	36	35	9	10	9.5	1	1	1	0	0	0	1	1	1
F23	p.m.	F	29	22	25.5	60	66	63	7	9	8	3	2	2.5	1	0	0.5	0	1	0.5
F24	p.m.	F	30	27	28.5	60	64	62	4	5	4.5	0	0	0	3	1	2	3	3	3
F25	p.m.	F	50	52	51	44	43	43.5	3	4	3.5	0	0	0	2	1	1.5	1	0	0.5
M1	p.m.	M	44	51	47.5	47	44	45.5	5	3	4	0	1	0.5	4	1	2.5	0	0	0
M2	p.m.	M	37	41	39	53	51	52	3	4	3.5	0	0	0	6	4	5	1	0	0.5
M3	p.m.	M	57	52	54.5	32	31	31.5	8	11	9.5	0	1	0.5	3	5	4	0	0	0
M4	p.m.	M	46	45	45.5	34	43	38.5	8	5	6.5	2	1	1.5	7	4	5.5	3	2	2.5
M5	p.m.	M	33	28	30.5	55	60	57.5	10	10	10	1	0	0.5	0	2	1	1	0	0.5
M6	p.m.	M	21	17	19	59	60	59.5	12	14	13	2	1	1.5	6	8	7	0	0	0
M7	p.m.	M	16	14	15	67	65	66	15	20	17.5	0	0	0	1	0	0.5	1	1	1
M8	p.m.	M	16	13	14.5	70	76	73	13	10	11.5	0	0	0	1	1	1	0	0	0
M9	p.m.	M	26	21	23.5	63	68	65.5	8	7	7.5	1	2	1.5	2	2	2	0	0	0
M10	p.m.	M	43	47	45	37	36	36.5	12	12	12	0	0	0	4	3	3.5	4	2	3
M11	p.m.	M	24	26	25	55	60	57.5	15	10	12.5	2	1	1.5	4	3	3.5	0	0	0
M12	p.m.	M	49	55	52	39	36	37.5	9	8	8.5	0	0	0	1	0	0.5	2	1	1.5
M13	p.m.	M	40	36	38	39	41	40	9	13	11	0	0	0	9	7	8	3	3	3
M14	p.m.	M	16	23	19.5	70	69	69.5	11	6	8.5	1	0	0.5	2	2	2	0	0	0
M15	p.m.	M	50	48	49	33	38	35.5	9	5	7	3	4	3.5	3	2	2.5	2	3	2.5
M16	p.m.	M	41	33	37	48	57	52.5	7	5	6	0	1	0.5	2	3	2.5	2	1	1.5
M17	p.m.	M	20	23	21.5	57	56	56.5	13	13	13	2	2	2	6	3	4.5	2	3	2.5
M18	p.m.	M	22	24	23	57	58	57.5	14	14	14	2	3	2.5	3	0	1.5	2	1	1.5
M19	p.m.	M	43	39	41	43	46	44.5	10	9	9.5	0	0	0	4	6	5	0	0	0
M20	p.m.	M	18	19	18.5	61	63	62	15	12	13.5	0	0	0	5	6	5.5	1	0	0.5
M21	p.m.	M	45	43	44	33	39	36	17	14	15.5	1	0	0.5	2	2	2	2	2	2
M22	p.m.	M	23	27	25	66	60	63	10	11	10.5	0	0	0	1	2	1.5	0	0	0
M23	p.m.	M	47	48	47.5	38	35	36.5	11	12	11.5	0	0	0	0	0	0	4	5	4.5
M24	p.m.	M	25	26	25.5	57	57	57	16	13	14.5	0	1	0.5	1	2	1.5	1	1	1
M25	p.m.	M	19	14	16.5	64	69	66.5	16	15	15.5	1	1	1	0	1	0.5	0	0	0

Figure 5. White blood cell differential reference ranges of 25 female (F) and 25 male (M) non-infected *Tamias amoenus*(p.m.) specimens including segmented neutrophil (S.N.), lymphocyte, monocyte, basophil, eosinophil and banded neutrophil (B.N.) percentages. Two differentials were performed for each individual and results for each count were averaged (avg).

#	Species	Sex	S.N.	2	avg	Lymphocytes	2	avg	Monocytes	2	avg	Basophils	2	avg	Eosinophils	2	avg	B.N.	2	avg
F1	t.a.	F	45	42	43.5	38	45	41.5	15	12	13.5	0	0	0	0	0	0	2	1	1.5
F2	t.a.	F	32	28	30	56	60	58	10	12	11	1	0	0.5	0	0	0	1	0	0.5
F3	t.a.	F	24	20	22	64	66	65	8	11	9.5	2	1	1.5	1	1	1	1	1	1
F4	t.a.	F	29	30	29.5	64	62	63	6	4	5	1	2	1.5	0	0	0	0	2	1
F5	t.a.	F	37	39	38	53	51	52	8	8	8	0	0	0	0	0	0	2	2	2
F6	t.a.	F	22	22	22	67	64	65.5	10	8	9	1	2	1.5	0	2	1	0	2	1
F7	t.a.	F	26	22	24	70	72	71	4	6	5	0	0	0	0	0	0	0	0	0
F8	t.a.	F	40	39	39.5	54	55	54.5	5	5	5	0	0	0	0	0	0	1	1	1
F9	t.a.	F	10	11	10.5	73	75	74	17	14	15.5	0	0	0	0	0	0	0	0	0
F10	t.a.	F	34	40	37	58	55	56.5	8	4	6	0	1	0.5	0	0	0	0	0	0
F11	t.a.	F	33	38	35.5	55	53	54	11	8	9.5	0	0	0	0	0	0	1	1	1
F12	t.a.	F	24	28	26	68	65	66.5	8	7	7.5	0	0	0	0	0	0	0	0	0
F13	t.a.	F	56	59	57.5	43	40	41.5	1	1	1	0	0	0	0	0	0	0	0	0
F14	t.a.	F	25	21	23	68	71	69.5	6	7	6.5	0	0	0	0	0	0	1	1	1
F15	t.a.	F	21	20	20.5	72	75	73.5	7	4	5.5	0	0	0	0	0	0	0	1	0.5
F16	t.a.	F	50	47	48.5	45	49	47	4	3	3.5	0	0	0	1	1	1	0	0	0
F17	t.a.	F	35	37	36	57	55	56	6	6	6	0	0	0	0	0	0	2	2	2
F18	t.a.	F	45	45	45	49	48	48.5	5	7	6	0	0	0	0	0	0	1	0	0.5
F19	t.a.	F	47	50	48.5	48	42	45	4	6	5	0	0	0	1	1	1	0	1	0.5
F20	t.a.	F	45	47	46	45	43	44	8	8	8	1	1	1	0	1	0.5	1	0	0.5
F21	t.a.	F	49	53	51	45	42	43.5	5	4	4.5	0	0	0	1	1	1	0	0	0
F22	t.a.	F	22	25	23.5	69	68	68.5	8	6	7	0	0	0	0	0	0	1	1	1
F23	t.a.	F	57	52	54.5	40	45	42.5	3	3	3	0	0	0	0	0	0	0	0	0
F24	t.a.	F	31	28	29.5	53	56	54.5	14	16	15	0	0	0	0	0	0	2	0	1
F25	t.a.	F	27	28	27.5	61	63	62	11	9	10	0	0	0	0	0	0	1	0	0.5
M1	t.a.	M	54	52	53	39	41	40	6	7	6.5	0	0	0	1	0	0.5	0	0	0
M2	t.a.	M	22	22	22	65	69	67	10	7	8.5	1	2	1.5	2	0	1	0	0	0
M3	t.a.	M	21	19	20	70	72	71	9	8	8.5	0	0	0	0	1	0.5	0	0	0
M4	t.a.	M	23	24	23.5	63	62	62.5	12	11	11.5	0	1	0.5	0	0	0	2	2	2
M5	t.a.	M	23	20	21.5	69	71	70	6	8	7	1	0	0.5	1	1	1	0	0	0
M6	t.a.	M	31	33	32	65	62	63.5	4	5	4.5	0	0	0	0	0	0	0	0	0
M7	t.a.	M	25	23	24	69	71	70	6	6	6	0	0	0	0	0	0	0	0	0
M8	t.a.	M	46	50	48	45	41	43	7	6	6.5	0	1	0.5	1	1	1	1	1	1
M9	t.a.	M	57	60	58.5	36	34	35	3	2	2.5	1	1	1	2	3	2.5	1	0	0.5
M10	t.a.	M	46	48	47	40	40	40	12	9	10.5	0	1	0.5	1	1	1	1	1	1
M11	t.a.	M	37	37	37	49	51	50	14	11	12.5	0	0	0	0	0	0	0	1	0.5
M12	t.a.	M	74	72	73	18	20	19	6	7	6.5	0	0	0	0	0	0	2	1	1.5
M13	t.a.	M	24	22	23	62	67	64.5	9	7	8	1	1	1	2	0	1	2	3	2.5
M14	t.a.	M	20	23	21.5	66	64	65	11	10	10.5	0	0	0	1	2	1.5	2	1	1.5
M15	t.a.	M	35	33	34	61	66	63.5	4	1	2.5	0	0	0	0	0	0	0	0	0
M16	t.a.	M	51	45	48	42	46	44	6	8	7	0	0	0	0	0	0	1	1	1
M17	t.a.	M	38	36	37	47	49	48	9	11	10	1	0	0.5	2	2	2	3	2	2.5
M18	t.a.	M	31	29	30	57	62	59.5	10	9	9.5	0	0	0	0	0	0	2	0	1
M19	t.a.	M	15	11	13	74	80	77	10	9	9.5	0	0	0	1	0	0.5	0	0	0
M20	t.a.	M	47	43	45	48	49	48.5	5	8	6.5	0	0	0	0	0	0	0	0	0
M21	t.a.	M	29	26	27.5	66	69	67.5	4	3	3.5	0	0	0	1	2	1.5	0	0	0
M22	t.a.	M	73	69	71	24	27	25.5	3	4	3.5	0	0	0	0	0	0	0	0	0
M23	t.a.	M	22	26	24	71	69	70	6	4	5	0	0	0	0	0	0	1	1	1
M24	t.a.	M	27	24	25.5	62	67	64.5	10	9	9.5	0	0	0	0	0	0	1	0	0.5
M25	t.a.	M	37	39	38	52	49	50.5	8	9	8.5	0	0	0	0	0	0	3	3	3

Figure 6. White blood cell differential reference ranges of 5 female (F) and 3 male (M) *Borrelia hermsii* infected *Callospermophilus lateralis* (c.l.) specimens including segmented neutrophil (S.N.), lymphocyte, monocyte, basophil, eosinophil and banded neutrophil (B.N.) percentages. Two differentials were performed for each individual and results for each count were averaged (avg).

#	Species	Sex	S.N.	2	avg	Lymphocytes	2	avg	Monocytes	2	avg	Basophils	2	avg	Eosinophils	2	avg	B.N.	2	avg
F1	c.l.	F	60	53	56.5	32	35	33.5	6	7	6.5	0	0	0	2	5	3.5	0	0	0
F2	c.l.	F	25	28	26.5	65	62	63.5	10	9	9.5	0	0	0	0	1	0.5	0	0	0
F3	c.l.	F	28	33	30.5	61	57	59	10	8	9	0	0	0	0	2	1	1	0	0.5
F4	c.l.	F	29	31	30	60	59	59.5	9	8	8.5	2	1	1.5	0	1	0.5	0	0	0
F5	c.l.	F	39	42	40.5	53	51	52	5	3	4	0	1	0.5	2	1	1.5	1	2	1.5
M1	c.l.	M	50	46	48	32	37	34.5	16	13	14.5	0	0	0	2	3	2.5	0	1	0.5
M2	c.l.	M	35	28	31.5	54	60	57	8	11	9.5	0	0	0	1	0	0.5	2	1	1.5
M3	c.l.	M	48	46	47	39	44	41.5	10	7	8.5	0	0	0	2	2	2	1	1	1

Figure 7. White blood cell differential reference ranges of 8 female (F), 7 male (M) and 2 unknown *Borrelia hermsii* infected *Peromyscus maniculatus* (p.m.) specimens including segmented neutrophil (S.N.), lymphocyte, monocyte, basophil, eosinophil and banded neutrophil (B.N.) percentages. Two differentials were performed for each individual and results for each count were averaged (avg).

#	Species	Sex	S.N.	2	avg	Lymphocytes	2	avg	Monocytes	2	avg	Basophils	2	avg	Eosinophils	2	avg	B.N.	2	avg
?1	p.m.	?	49	48	48.5	43	43	43	2	3	2.5	1	1	1	2	2	2	3	3	3
?2	p.m.	?	39	43	41	49	46	47.5	6	4	5	0	0	0	0	0	0	6	7	6.5
F1	p.m.	F	49	52	50.5	43	40	41.5	4	5	4.5	0	0	0	3	2	2.5	1	1	1
F2	p.m.	F	17	14	15.5	80	84	82	3	2	2.5	0	0	0	0	0	0	0	0	0
F3	p.m.	F	25	24	24.5	65	67	66	3	3	3	1	0	0.5	5	5	5	1	1	1
F4	p.m.	F	21	18	19.5	72	77	74.5	5	4	4.5	0	0	0	2	1	1.5	0	0	0
F5	p.m.	F	20	17	18.5	73	75	74	5	6	5.5	1	0	0.5	0	0	0	1	2	1.5
F6	p.m.	F	43	38	40.5	48	51	49.5	1	5	3	2	1	1.5	5	4	4.5	1	1	1
F7	p.m.	F	18	21	19.5	76	73	74.5	2	3	2.5	3	2	2.5	0	0	0	1	1	1
F8	p.m.	F	24	25	24.5	68	70	69	7	5	6	0	0	0	1	0	0.5	0	0	0
M1	p.m.	M	39	42	40.5	58	55	56.5	0	1	0.5	0	0	0	0	0	0	3	2	2.5
M2	p.m.	M	15	11	13	75	76	75.5	6	9	7.5	1	2	1.5	3	2	2.5	0	0	0
M3	p.m.	M	42	42	42	48	45	46.5	6	8	7	2	2	2	0	0	0	2	3	2.5
M4	p.m.	M	19	24	21.5	67	65	66	8	6	7	1	1	1	1	1	1	4	3	3.5
M5	p.m.	M	47	48	47.5	44	44	44	2	2	2	0	0	0	2	3	2.5	5	3	4
M6	p.m.	M	11	13	12	82	80	81	2	3	2.5	0	1	0.5	5	2	3.5	0	1	0.5
M7	p.m.	M	24	21	22.5	71	73	72	1	3	2	2	1	1.5	2	2	2	0	0	0

Figure 8. White blood cell differential reference ranges of 15 female (F) and 12 male (M) *Borrelia hermsii* infected *Tamias amoenus* (t.a.) specimens including segmented neutrophil (S.N.), lymphocyte, monocyte, basophil, eosinophil and banded neutrophil (B.N.) percentages. Two differentials were performed for each individual and results for each count were averaged (avg).

#	Species	Sex	S.N.	2	avg	Lymphocytes	2	avg	Monocytes	2	avg	Basophils	2	avg	Eosinophils	2	avg	B.N.	2	avg
F1	t.a.	F	32	36	34	57	53	55	9	9	9	1	1	1	1	0	0.5	0	1	0.5
F2	t.a.	F	13	11	12	80	84	82	5	3	4	1	0	0.5	1	2	1.5	0	0	0
F3	t.a.	F	17	19	18	77	73	75	6	7	6.5	0	0	0	0	1	0.5	0	0	0
F4	t.a.	F	23	19	21	67	69	68	10	12	11	0	0	0	0	0	0	0	0	0
F5	t.a.	F	39	39	39	55	53	54	5	8	6.5	0	0	0	0	0	0	1	0	0.5
F6	t.a.	F	27	31	29	65	62	63.5	8	7	7.5	0	0	0	0	0	0	0	0	0
F7	t.a.	F	31	34	32.5	63	58	60.5	6	7	6.5	0	0	0	0	0	0	0	1	0.5
F8	t.a.	F	21	16	18.5	69	74	71.5	9	9	9	0	0	0	0	0	0	1	1	1
F9	t.a.	F	14	16	15	77	70	73.5	9	12	10.5	0	0	0	0	2	1	0	0	0
F10	t.a.	F	46	47	46.5	48	46	47	6	7	6.5	0	0	0	0	0	0	0	0	0
F11	t.a.	F	21	21	21	68	71	69.5	11	7	9	0	1	0.5	0	0	0	0	0	0
F12	t.a.	F	22	18	20	66	70	68	11	12	11.5	0	0	0	0	0	0	1	0	0.5
F13	t.a.	F	40	47	43.5	56	51	53.5	4	2	3	0	0	0	0	0	0	0	0	0
F14	t.a.	F	20	17	18.5	69	73	71	11	10	10.5	0	0	0	0	0	0	0	0	0
F15	t.a.	F	36	40	38	58	55	56.5	6	5	5.5	0	0	0	0	0	0	0	0	0
M1	t.a.	M	28	24	26	65	65	65	7	9	8	0	0	0	0	0	0	0	2	1
M2	t.a.	M	39	42	40.5	53	52	52.5	5	4	4.5	0	0	0	2	1	1.5	1	1	1
M3	t.a.	M	48	45	46.5	46	49	47.5	6	6	6	0	0	0	0	0	0	0	0	0
M4	t.a.	M	14	18	16	81	77	79	4	4	4	1	1	1	0	0	0	0	0	0
M5	t.a.	M	42	40	41	51	54	52.5	7	6	6.5	0	0	0	0	0	0	0	0	0
M6	t.a.	M	38	32	35	59	60	59.5	3	6	4.5	0	0	0	0	0	0	0	2	1
M7	t.a.	M	60	59	59.5	39	41	40	1	0	0.5	0	0	0	0	0	0	0	0	0
M8	t.a.	M	29	30	29.5	58	54	56	13	15	14	0	0	0	0	1	0.5	0	0	0
M9	t.a.	M	28	25	26.5	66	69	67.5	6	6	6	0	0	0	0	0	0	0	0	0
M10	t.a.	M	34	30	32	65	68	66.5	1	2	1.5	0	0	0	0	0	0	0	0	0
M11	t.a.	M	54	54	54	37	35	36	9	10	9.5	0	0	0	0	0	0	0	1	0.5
M12	t.a.	M	20	16	18	74	76	75	6	8	7	0	0	0	0	0	0	0	0	0

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