Journal of the American Association for Laboratory Animal Science
Copyright 2020
by the American Association for Laboratory Animal Science

Vol 59, No 3
May 2020
Pages 275–281

Hematologic Values of Jamaican Fruit Bats (*Artibeus jamaicensis*) and the Effects of Isoflurane Anesthesia

Alyssa A Strumpf,[1] Ashley Malmlov,[1] Jessica D Ayers,[2] Tony Schountz,[1] and Lon V Kendall[1,2,*]

Jamaican fruit bats (*Artibeus jamaicensis*) are used as an animal model for several viruses, including Middle East respiratory syndrome virus, dengue virus, Zika virus, and Tacaribe virus. However, despite ongoing studies regarding these pathogens, little is known regarding the bats' normal physiology. In this study, phlebotomy of the propetagial (cephalic) vein was performed to establish baseline hematologic parameters in an apparently healthy, captive population of Jamaican fruit bats. Furthermore, we compared results from physically restrained and isoflurane-anesthetized bats. Our findings indicate significant increases in WBC count, lymphocytes, and monocytes in the anesthetized bats. However, RBC and platelet parameters were not different between the 2 groups. This information on the normal hematologic parameters of Jamaican fruit bats, adds to our overall understanding of the normal physiology of this species, and expands our knowledge on bat species in general.

Abbreviation: JFB, Jamaican fruit bats

DOI: 10.30802/AALAS-JAALAS-19-000056

According to the US Department of Interior, more than 1300 species of bats are classified into 18 distinct families, comprise more than 20% of living mammal species, and reside nearly worldwide except for the Arctic and Antarctic.[27,31] Bats commonly inhabit both temperate and tropical regions and colonize numerous roosts, from barns and attics to trees and rock crevices and essentially span from deep within tropical forests to the backyards of suburban neighborhood. Bats also vary in their dietary requirements, including nectivorous, insectivorous, frugivorous, and vampire species. Bats play pivotal roles both ecologically and economically and are valuable members of the animal community. They help control insect populations and pollinate several plant species of importance for human food consumption and medicinal therapies.[31] Furthermore, analyses in 2011 showed that in North America alone, the loss of bats— primarily due to white-nose syndrome and wind turbines—could lead to more than $3.7 billion loss annually, making it more imperative to protect bats and their habitats.[2] However, despite their overall benefits to society, bats have also been found to serve as reservoirs for high-risk human pathogens.[4]

Bats belong to the order Chiroptera, which has 2 historic suborders: Megachiroptera and Microchiroptera. Each suborder was divided originally based primarily on its geographic distribution and anatomic features.[1,3] Recent phylogenetic data supports a different classification scheme, highlighting a paraphyletic ancestry over the historically supported monophyletic taxonomic organization.[28,30] Moreover, this information has led to the classification of bats into 2 current suborders: Yinpterochiroptera (Old World bats) and Yangochiroptera (Old and New World bats), which includes Jamaican fruit bats (JFB, *Artibeus jamaicensis*).[28,30]

A great deal of information is available regarding captive management, diet, and even the numerous pathogens of fructivorous bats.[7] However, knowledge gaps regarding bat immune responses and viral pathogenesis remain, hindering what is known about disease pathogenesis and why bats can harbor deadly human pathogens without succumbing to disease themselves.[3] Disease transmission occurs sporadically from bats to humans and has been directly observed in only a few of these known pathogens: rabies and related lyssaviruses and 2 henipaviruses, Hendra and Nipah.[4] Likely due to multifactorial issues including limited funding, habitat loss, and sporadic emergence, more emphasis of research is placed on diseases that greatly affect human health compared with that of bats themselves.[3,4]

Over the past few decades, bats have become recognized as a public health concern regarding the virus–host interactions for serious and sometimes fatal zoonotic diseases. Bats are reservoirs for or suspected reservoir hosts of filoviruses, henipaviruses, coronaviruses, and lyssaviruses, some of which cause significant disease in humans and livestock.[4,23] JFB have been used more than any other bat species to study viral infections, including those of Middle East respiratory syndrome coronavirus, dengue virus, Zika virus, Tacaribe virus, and rabies virus.[5,11,12,17,18,19,22,25] Despite the growing importance of this species for zoonotic virus disease research, virtually nothing is known about the basic physiology of JFB.

Methods for determining normal physiologic parameters of bats, either using captive colonies or wild populations, are limited. Most researchers conducting bat studies typically follow catch-and-release protocols, without additional diagnostic testing beyond what is needed for the study. Although

Received: 16 Apr 2019. Revision requested: 03 Jul 2019. Accepted: 07 Oct 2019.
[1]Department of Microbiology, Immunology, and Pathology and [2]Laboratory Animal Resources, Colorado State University, Fort Collins, Colorado
[*]Corresponding author. Email: lon.kendall@colostate.edu

additional diagnostic samples may be collected at this time, they are frequently not, due to minimal sample volumes or lack of validated testing modalities. Captive populations allow continuous observation and study of normal behavior and physiology over an extended time in a controlled environment, thus potentially providing a more comprehensive understanding of the species and extrapolate information to the wild population.

Hematology is frequently used for monitoring in veterinary medicine and provides an important tool to detect health abnormalities and make comparisons among different species and—in research—between study cohorts. Hematologic parameters can help interpret population health as well as potential diseases not yet explored in either captive or wild populations of bats.

In our experience, JFB are a species that can be restrained without anesthesia, which substantially facilitates various procedures, such as blood collection. However, the ease of using anesthetized research subjects is beneficial, reducing stress for subjects and facilitating collection procedures. Previous studies performed in other species, including dogs, guinea pigs, and fructivorous bats, revealed significant differences in hematology parameters between anesthetized and unanesthetized animals.[15,29,33] Our aims for the current study were 1) to establish baseline parameters for a captive, healthy population of JFB and 2) to determine whether differences in hematologic parameters occur in the same population when blood collection is performed on manually restrained compared with isoflurane-anesthetized bats. Furthermore, the values obtained from our study can be used for comparison between healthy and infected JFB as well as other species with known hematologic parameters, to identify physiologic differences among bats.[8,14,15,21,22,26,32,34]

Materials and Methods

Animals. The JFB colony at Colorado State University was originally founded in 2016 with bats from a captive population at another institution that was maintained as a closed breeding colony for more than 10 y. Approximately 150 JFB were cohoused with approximately 30 Seba fruit-eating bats (*Carollia perspicillata*) in a free-flight room measuring 5.8 × 3 × 2.7 m. Roosting material was hung from the ceiling in various places throughout the room, and black drapes were positioned along the walls for additional roosting. Ambient temperature was maintained between 20 and 25 °C, with humidity ranging between 50% and 70%, and a 12:12-h dark:light cycle, through use of a computer-controlled system. Female and male bats were cohoused. Bats were fed once daily from multiple feeding trays containing a combination of fruits and grueled Teklad primate chow (Envigo, Indianapolis, IN), consisting of molasses, nonfat dry milk, and cherry gelatin.[7] To stimulate foraging behavior and provide enrichment, additional fruit was hung around the room. Water was provided as needed. Samples were collected without regard to sex and were characterized as a single population. New mothers with unweaned pups—but not pregnant females—were excluded from blood collection. All experimental procedures were approved by the IACUC; our institution is an AAALAC-accredited facility.

Unanesthetized blood collection. To determine hematologic parameters in unanesthetized bats, 28 bats from the free-flight room were captured on 2 consecutive days (n = 16 and n = 12, respectively) for phlebotomy. Awake bats were caught by using either sturdy leather gloves or a butterfly net and were appropriately restrained to allow full access to the wings for blood collection (Figure 1). Prior to blood withdrawal, wings were held approximately 30 cm from an infrared heat lamp (Portable Luminaire, 250A bulb) for 30 s to 2 min to induce vasodilation. By using this procedure, the vein was clearly visible. Blood was withdrawn from the propetagial (cephalic) vein. A 26-gauge, 20-mm intradermal needle (Precision Guide Needle, Becton Dickinson, Franklin Lakes, NJ) was used to make a small laceration in the vein, to allow free flow of blood (Figure 2). Heparinized microhematocrit capillary tubes (VWR Scientific, Broomall, PA) were used to collect blood. We filled 3 or 4 microhematocrit tubes to collect a total of 180 to 240 µL of blood from each bat. Blood was transferred from the microhematocrit tubes to EDTA microtubes (1.6 mg EDTA/mL, Sarstedt, Numbrecht, Germany) for submission to the Colorado State University Veterinary Diagnostic Laboratory; all samples were kept on ice before and during transport to the diagnostic lab. After blood collection, 2×2-in. gauze (AmensourceBergen, Chesterbrook, PA) was held with moderate pressure on the venipuncture site to initiate clotting. Bats were released into a small, wire cage lined with a linen cloth to allow them to hang. Fresh chopped fruit was provided at the bottom of the cage after blood collection. Bats remained cohoused in cages for 24 h after blood collection and were observed for any adverse signs, including lethargy, weakness, inappetence, and death. After this observation period, they were removed from the colony for an experimental study.

Anesthetized blood collection. Eighteen days after unanesthetized blood collection, a different cohort of bats (n = 15) was caught and sampled as described earlier. Anesthesia was induced by using a rat-sized oxygen mask. Initially, bats received approximately 0.5 to 1.0 L/min O_2 with 3% to 5% isoflurane (VetOne, Boise, ID). Anesthesia was deemed adequate by easy extension and flexion of the wing, without any voluntary movement. Bats were maintained on 1.5% to 3% isoflurane and 0.5 to 1.0 L/min O_2 as necessary to provide an adequate plane of anesthesia throughout the procedure. Blood was collected as described earlier. Isoflurane was discontinued immediately after blood collection, and each bat remained on 0.5 to 1.0 L/min of O_2 for 60 to 120 s. Total time under anesthesia ranged from 3 to 10 min, depending on the rapidity of blood collection. Bats were held with a handling glove until recovery and then released into a small, wire cage lined with a linen cloth for hanging. They were monitored for the next 24 h, as described earlier and then returned to the colony.

Hematology. Immediately after collection, blood samples were transported on ice from the animal facility to the Colorado State University Veterinary Diagnostic Laboratory. Clinical pathology personnel performed CBC counts on each sample by using an automated hematology system (Advia 120, Siemens, Malvern, PA), and results were processed by using canine reference ranges.

Statistical analysis. All statistics were performed by using Prism 8.0.1 (GraphPad Software, La Jolla, CA). Means, standard deviations, and ranges were calculated for all hematology parameters of the 28 unsedated and 15 anesthetized animals, and 95% confidence intervals were generated also. Pairwise comparisons were performed for each hematologic parameter. Normality was determined by using the Shapiro–Wilk test; the Welch t test was used when the data were normally distributed (lymphocyte percentage, monocyte percentage, granulocyte percentage, MCV, RDW percentage, Hgb, MCHC, MCH, and RBC), whereas the Mann–Whitney rank sum test was used when data were not normally distributed (WBC, lymphocytes, monocytes, granulocytes, Hct, RDW actual, platelet count, and MPV). A P value less than 0.05 was considered statistically sig-

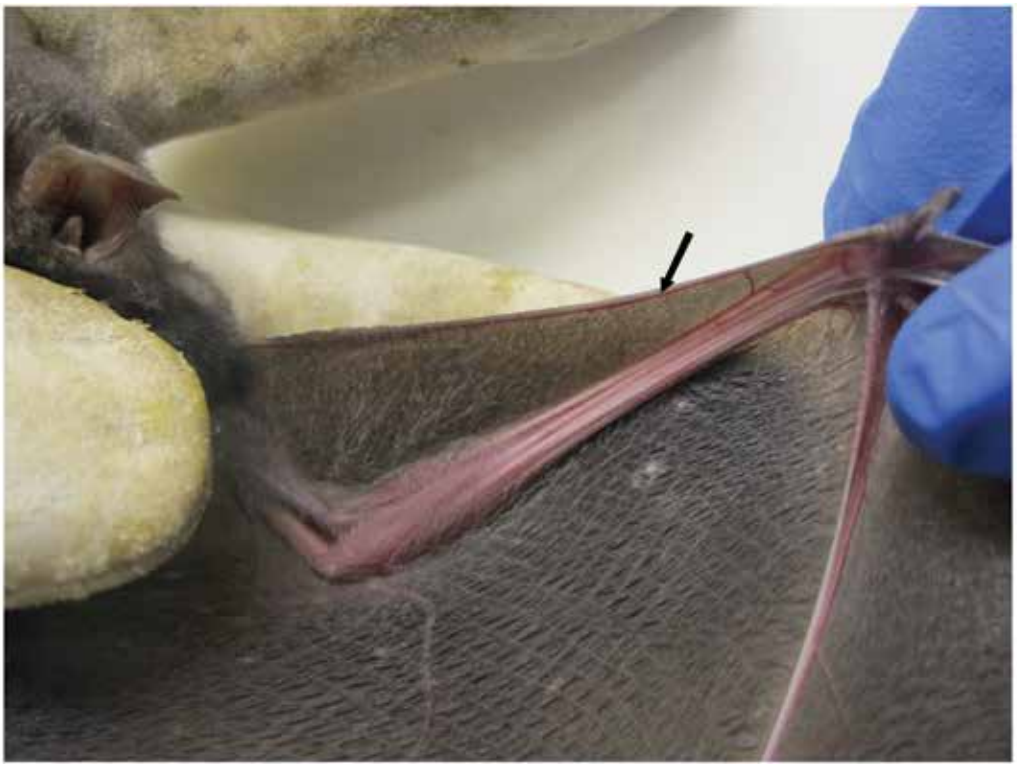

Figure 1. Extended wing of a Jamaican fruit bat, highlighting the propetagial (cephalic) vein (arrow). Although the vein is initially small and difficult to visualize, dilation after using a heat lamp enables the vein to be visualized appropriately for phlebotomy.

nificant. Outliers were determined by using the ROUT method, with a maximum false discovery rate of 0.1%. Age, sex, body condition, and reproductive status were not considered in the parameters assessed.

Results

To develop baseline hematologic parameters and elucidate differences in these values according to the presence or absence of anesthesia, we collected blood from 2 groups of JFB, unsedated and anesthetized. Blood was collected successfully from all bats in this study. One bat was found dead at 24 h after collection; the remainder of the bats had no adverse events at the 24-h postcollection observation. The WBC measurements of physically restrained and anesthetized bats differed significantly, with lower overall WBC counts and lymphocyte counts in unsedated bats compared with the anesthetized group (Table 1); RBC and platelet parameters did not change with anesthesia (Table 1). We then compared our findings for JFB with established baseline reference intervals for several other species of bats[8,14,15,21,22,26,32,34] (Table 2).

Discussion

Despite ongoing studies in bats, primarily focused on zoonotic infectious agents that can cause human epidemic or pandemic infections, little is known about the normal physiology in all bat species. Furthermore, few captive bat populations are available for research purposes. Here, the current study had 2 aims: 1) to determine baseline hematologic values for an apparently healthy, captive population of JFB and 2) to compare the hematologic values of physically restrained (unsedated) and anesthetized bats.

First, we evaluated and compared baseline parameters between unsedated and anesthetized JFB. We collected blood samples from a group of bats (n = 28) to define parameters for a normal, captive population of JFB. Several other studies have established baseline reference intervals for various species of bats: gray-headed flying fox (*Pteropus poliocephalus*), Christmas Island flying fox (*Pteropus melanotus natalis*), island flying fox (*Pteropus hypomelanus*), Indian flying fox (*Pteropus giganteus*), black flying fox (*Pteropus alecto*), straw-colored fruit bat (*Eidolon helvum*), Egyptian fruit bat (*Rousettus aegpytiacus*), and Daubenton bat (*Myotis daubentonii*).[8,14,15,21,22,26,32,34] Despite having baseline reference intervals for various bat species, comparing previous findings with our current study is difficult, primarily due to the varied ecology of individual species, with some being insectivorous and others frugivorous. JFB are small in stature, usually weighing between 40 and 60 g, which limits the ease and volume of blood collection.[24] We did not consider both age and sex as factors in hematology values, whereas other studies collected specific information for females, males, juveniles, and adults and, in some cases, lactating compared with nonlactating females and wild-caught compared with captive bats. Moreover, the parameters assessed in each study varied. Given these considerations, average RBC parameters appear to be lower in *E. helvum*, *M. daubentonii*, *P. alecto*, *P. hypomelanus*, *P melanotus natalis*, and *P. poliocephalus* as compared with JFB, whereas *R. aegyptiacus* had overall higher RBC parameters.[8,14,15,22,26,32,34] WBC parameters were much more variable between species. *M. daubentonii*, *P. hypomelanus*, *P. melanotus natalis*, and *P. poliocephalus* appeared to have higher lymphocyte-to-granulocyte ratios, much like JFB, whereas *P. alecto* and *P. giganteus* had higher granulocyte-to-lymphocyte ratios and *E. helvum* had nearly equivalent numbers of granulocytes and lymphocytes (Table 2).[8,14,15,21,22,26,34] A 2011 hematologic survey of more than 255 bats assessed Hct and total and differential WBC counts.[25] Although those data were collected from free-ranging bats, rather than from a captive population, the results amassed for JFB previously[25] were similar to those found in our study. The

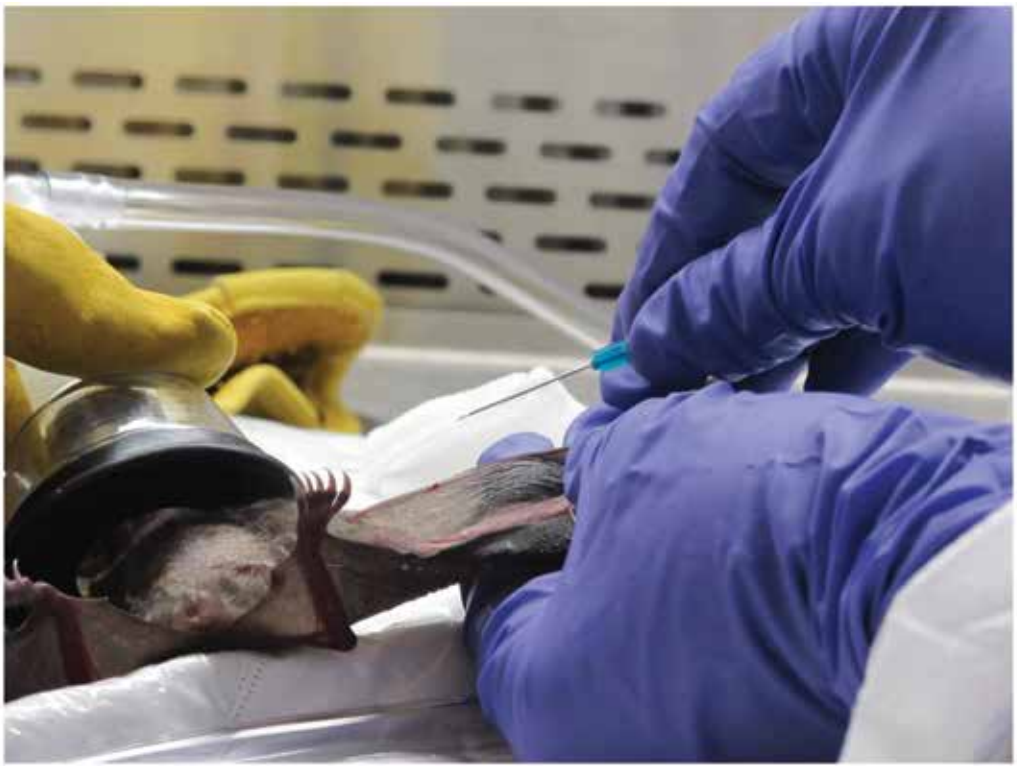

Figure 2. Phlebotomy of the propetagial (cephalic) vein of a Jamaican fruit bat by using a 26-gauge, 20-mm intradermal needle. This bat was in the anesthetized group and is being restrained under isoflurane gas delivered through a rat-sized mask.

Table 1. Hematologic reference intervals for clinically normal, captive, physically restrained (n = 28) compared with anesthetized (n = 15) adult Jamaican fruit bats

	Mean ± 1 SD			Range		95% CI	
	Physically restrained	Anesthetized	P	Physically restrained	Anesthetized	Physically restrained	Anesthetized
WBC, 10^3/µL	4.68 ± 2.36	7.09 ± 2.01	0.001	2.2–11.8	3.8–10.1	3.77–5.6	5.97–8.2
Lymphocytes, 10^3/µL	2.56 ± 1.99	4.21 ± 1.11	0.001	0.9–10.2	2.2–6.1	1.79–3.34	3.59–4.82
Monocytes, 10^3/µL	0.49 ± 0.19	0.63 ± 0.17	0.01	0.3–1.0	0.4–0.9	0.42–0.56	0.54–0.73
Granulocytes, 10^3/µL	1.63 ± 0.73	2.25 ± 1.24	0..13	0.6–3.4	0.7–5.2	1.35–1.91	1.56–2.93
Lymphocytes, %	51.92 ± 12.76	60.66 ± 10.34	0.02	34.1–86.8	37.7–81.1	46.97–56.87	54.93–66.39
Monocytes, %	9.9 ± 2.05	8.21 ± 1.07	0.001	4.7–12.7	6.5–10.4	9.11–10.69	7.62–8.8
Granulocytes, %	38.18 ± 11.26	31.13 ± 9.88	0.04	8.5–55.2	12.4–52.9	33.82–42.55	25.65–36.6
Hct, %	53.43 ± 2.54	52.43 ± 2.63	0.17	47.2–59.8	48.4–57.1	52.44–54.41	50.97–53.88
MCV, fL	39.38 ± 1.14	39.46 ± 1.67	0.87	37.3–41.1	37–42.8	38.94–39.82	38.54–40.38
RDW, absolute	22.83 ± 0.68	22.67 ± 0.53	0.35	21.4–24.0	22–24.1	22.56–23.09	22.37–22.96
RDW, %	20.44 ± 0.83	20.24 ± 0.91	0.48	19.5–22.2	18.9–21.7	20.12–20.76	19.74–20.74
Hgb, g/dL	18.73 ± 0.91	18.25 ± 0.85	0.09	16.7–21.3	17.1–20	18.38–19.08	17.77–18.72
MCHC, g/dL	35.07 ± 0.76	34.85 ± 0.65	0.31	33.4–36.5	33.7–36.3	34.78–35.37	34.49–35.21
MCH, g/dL	13.8 ± 0.372	13.73 ± 0.54	0.66	13.2–4.4	13–14.9	13.65–13.94	13.43–14.02
RBC, 10^6/µL	13.56 ± 0.73	13.31 ± 0.96	0.37	11.96–15.4	11.96–15.43	13.28–13.85	12.77–13.84
Platelets, 10^3/µL	102.5 ± 18.55	123.5 ± 54.47	0.12	76–163	62–278	95.27–109.7	93.37–153.7
MPV, fL	6.16 ± 0.18	6.06 ± 0.21	0.12	5.8–6.4	5.7–6.4	6.09–6.24	5.94–6.18

P values between physically restrained and anesthetized bats determined by using either a paired t test or the Wilcoxon rank-sum test.

differences in the means and ranges of hematologic parameters of these variety of bat species illustrate the need to have baseline parameters for each species of bat. In addition, the above data obtained from ref 25 and other studies [8,14,15,22,26,32,34] are not inclusive to all hematologic parameters assessed in various bat species.

Our study evaluated differences in hematologic values when phlebotomy was performed on unsedated compared with anesthetized bats. The data revealed significant differences in WBC (specifically, lymphocytes and monocytes) but not RBC or platelet parameters (Table 1). Overall WBC and lymphocyte counts were lower in unsedated bats compared with the anesthetized group (Table 1). A previous study in guinea pigs compared hematologic and biochemical parameters from animals that were physically restrained, anesthetized with isoflurane, or anesthetized with an injectable regimen.[33] WBC counts were

Table 2. Comparison of hematologic parameters (mean [range], when available) from various species of bats when phlebotomy was performed on awake, physically restrained animals

	A. jamaicensis	*P. poliocpehalus*[7]	*P. melanotus natalis*[13]	*P. hypomelanus*[14]	*P. alecto*[21]	*E. helvum*[25]	*R. aegypticus*[31]	*M. daubentoni*[33]
RBC, $10^6/\mu L$	13.56 (11.96–15.40)	9.3 (7.5–10.9)	—	8.25	9.1 (7.6– 12.6)	9.47 (7.74–10.89)	14.87	10.7
Hct, %	53.4 (47.2–59.8)	37.0 (30.0–42.0)	40.7 (36.0–44.0)	46.6	47.0 (39.0–62.0)	42.7 (33.3–51.0)	57.4	46.5
Hgb, g/dL	18.7 (16.7–21.3)	15.4 (12.7–17.7)	—	15.95	16.4 (13.6–21.8)	14.9 (12.0–16.0)	15.0	15.0
WBC, $10^3/\mu L$	4.7 (2.2–11.8)	5.4 (0.0–10.8)	8.4 (2.6–13.2)	16.55	6.0 (2.5–22.0)	3.2 (1.2–7.3)	11.45	3.36
Lymphocytes, $10^3/\mu L$	2.6 (0.9–10.2)	1.7 (0.0–5.1)	4.58 (0.91–7.66)	10.65	1.7 (0.3–12.1)	1.70 (0.64–4.03)	—	2.07
Granulocytes, $10^3/\mu L$	1.6 (0.6–3.4)	3.5 (0.5–8.6)	3.74 (1.69–5.71)	4.70	3.6 (0.9–12.5)	1.36 (0.29–6.0)	—	1.23

Values have been adapted to compare adults of each species with our population of bats. Units have been adapted for consistency.

significantly higher in the injectable anesthesia group compared with the unsedated group, with a significant difference in heterophils.[33] The authors attributed the significant changes between the unsedated animals and the injectable anesthesia group to a stress leukogram, but found no significant differences between the unsedated and isoflurane groups. Stress is often a component of animal behavior during physical restraint and is observable on a leukogram as a glucocorticoid-induced response. A stress leukogram is typically characterized by mature neutrophilia and lymphopenia, with lymphopenia being the more consistent change. These WBC changes in the circulating blood are primarily affected by the release of endogenous corticosteroids during the stressful episode. Stress leukograms can occur in all animals but are most common and pronounced in dogs.[10] Our results revealed overall higher WBC parameters in the anesthetized group compared with the unsedated group of bats, but the values do not follow the common trend for a stress leukogram (Table 1). Furthermore, given the similarities in WBC parameters shown previously,[25] future studies comparing unsedated bats with those anesthetized by using injectable anesthetics would be beneficial to elucidate whether a stress leukogram appears. Other studies involving rats and mice have compared hematologic parameters in unsedated and anesthetized (isoflurane gas) groups, both of which have shown no significant changes in the WBC parameters.[6,16] Unlike our findings, the previous bat study[25] found significant differences in the Hct, showing a significantly decreased Hct in the isoflurane group compared with the unsedated animals. Although the authors initially hypothesized that this effect could have been due to sequential blood draws and the development of anemia, this explanation was ruled out given other findings comparing isoflurane inhalant to injectable anesthetics.[33] In addition, studies in various other mammals have found similar changes in RBC parameters, which can be attributed to the effects of isoflurane, particularly splenic sequestration due to splenic vasodilation.[6,15,20,29]

Isoflurane is a general inhalant anesthetic that is often used in veterinary medicine because of its ease of use and minimal adverse effects in otherwise healthy patients. Although the mechanism of action is still not completely understood, inhalant anesthetics induce reversible, dose-related unresponsiveness to noxious stimuli—that is, a state of general anesthesia. Physiologically, isoflurane has been shown to cause variable effects in many organ systems, most notably the cardiovascular system. The anesthetic alters cardiac output, typically causing a decrease in stroke volume and therefore an increase in heart rate. Furthermore, the decrease in cardiac output reduces total blood flow. Subsequently, isoflurane may also play a role in redistributing blood flow to tissues.[13] The vasodilatory effects of inhalant anesthesia may play a crucial role in lowering RBC parameters due to splenic sequestration.[13] Despite the differences in hematologic parameters in various other mammals of similar studies,[15,33] we did not observe these differences in our sample population of bats. In our study, bats were only under isoflurane anesthesia for a short period of time, which could have influenced its physiologic effects. Future studies are needed to determine whether physiologic differences are dependent on time under inhalant anesthesia. Furthermore, there may be unknown physiologic differences among JFB such that they do not demonstrate the same hematologic alterations under anesthesia as observed in other species.

Although we were successful in establishing baseline parameters for CBC counts in a captive population of JFB, our study has limitations. Three outliers within the dataset—one for total WBC and 2 for lymphocyte counts in the physically restrained group—were included in the analysis. These outliers could be due to random variation among the bats or to underlying disease, such as subclinical infection, that was not evident during our initial physical examination. When these outliers are removed, the WBC count (mean ± 1 SD) becomes $4.4 \pm 1.9 \times 10^3/\mu L$, the lymphocyte count becomes $2.1 \pm 1.1 \times 10^3/\mu L$, and the values for the physically restrained bats remain significantly different ($P < 0.01$) compared with the anesthetized group's values.

Our captive population was established in 2016; however, we are unaware of the age of each individual bat. We attempted to exclude bats that appeared to be juveniles from the study, and it was not possible to assess age as a potential contributing factor for our results, as was assessed in previous studies of other bat species.[8,14,21,34] Furthermore, we did not separate bats according to sex or reproductive status. Hematologic values likely differ between sexes of bats; however, we did not evaluate sex-specific data as a subset, because many veterinary reference ranges are inclusive of both sexes.

Given their small size and difficult access to venipuncture sites, obtaining appropriate amounts of blood to perform CBC counts of bats can be difficult.[9,22] We were able to collect enough blood to perform CBC counts successfully; however, we were unable to collect enough blood during the same phlebotomy episode to also perform a biochemistry panel. Further investigation is needed to collect information regarding the biochemistry

parameters in JFB to provide a complete health panel in addition to hematology. Within the 24-h postcollection period, one bat was found dead. A necropsy was not performed; given the timing relative to blood collection, we presumed that death was due to stress associated with capture and confinement, profound anemia from blood collection, or an unknown event. Given the increased likelihood of stress and anemia compared with an unknown event, care needs to be taken to prevent excessive collection of blood in a single episode, which might result in anemia or death, in severe cases. One way to avoid this situation is to use another collection site, such as the interfemoral vein.[9]

We found significant differences in hematologic parameters between physically restrained (unsedated) and anesthetized bats, particularly WBC parameters, showing lower total WBC, lymphocyte, and monocyte counts in physically restrained bats. The hematologic values of physically restrained and anesthetized bats should be compared with caution, and appropriate controls in the experimental design should be used to account for this potential variable. These baseline hematologic parameters, which were obtained from a captive population of JFB, will further aid in the evaluation of their health status.

Acknowledgments

We thank Drs Whitney McGee, Alexa Personett, and Brian Smith for contributions toward data collection and Ben Prytherch for assistance with statistical analysis of data. We also thank the Colorado State University Laboratory Animal Resources department for technical and husbandry support. Support of the colony was provided by NIAID grant no. R01AI134768. Support for this study was provided by the Office of the Vice President for Research and the Department of Microbiology, Immunology, and Pathology at Colorado State University.

References

1. **Boonman A, Bar-On Y, Cvikel N, Yovel Y.** 2013. It's not black or white—on the range of vision and echolocation in echolocating bats. Front Physiol **4:**1–12. https://doi.org/10.3389/fphys.2013.00248.
2. **Boyles JG, Cryan PM, McCracken GF, Kunz TH.** 2011. Economic importance of bats in agriculture. Science **332:**41–42. https://doi.org/10.1126/science.1201366.
3. **Buckles EL.** 2015. Chiroptera (Bats), Chapter 35. p 281–290. In: Miller RE, Fowler ME, editors. Fowler's zoo and wild animal medicine, vol 8. St Louis (MO): Elsevier Saunders.
4. **Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T.** 2006. Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev **19:**531–545. https://doi.org/10.1128/CMR.00017-06.
5. **Cogswell-Hawkinson A, Bowen R, James S, Gardiner D, Calisher CH, Adams R, Schountz T.** 2012. Tacaribe virus causes fatal infection of an ostensible reservoir host, the Jamaican fruit bat. J Virol **86:**5791–5799. https://doi.org/10.1128/JVI.00201-12.
6. **Deckardt K, Weber I, Kaspers U, Hellwig J, Tennekes H, van Ravenzwaay B.** 2007. The effects of inhalation anaethetics on common clinical pathology parameters in laboratory rats. Food Chem Toxicol **45:**1709–1718. https://doi.org/10.1016/j.fct.2007.03.005.
7. **Dempsey JL.** [Internet]. 2004. Fruit bats: nutrition and dietary husbandry. [Cited 21 June 2019]. Available at: https://nagonline.net/834/fruit-bats-nutrition-dietary-husbandry/.
8. **Edson D, Field H, McMichael L, Mayer D, Martin J, Welbergen J, McLaughlin A, Huth L, Kristoffersen J, Tsoukalas G, Kirkland P.** 2018. Hematology, plasma biochemistry, and urinalysis of free-ranging grey-headed flying foxes (*Pteropus poliochephalus*) in Australia. J Zoo Wildl Med **49:**591–598. https://doi.org/10.1638/2017-0126.1.
9. **Eshar D, Weinberg M.** 2010. Venipuncture in bats. Lab Anim (NY) **39:**175–176. https://doi.org/10.1038/laban0610-175.
10. **Gaunt SD.** 2004. Interpretation of the leukogram, Chapter 10. p 38–44. In: Cowell RL, editors. Veterinary clinical pathology secrets. Irving (TX): Hanley and Belfus.
11. **Gerrard DL, Hawkinson A, Sherman T, Modahl CM, Hume G, Campbell CL, Schountz T, Frietze S.** 2017. Transcriptomic signatures of tacaribe virus-infected Jamaican fruit bats. MSphere **2:**1–15. https://doi.org/10.1128/mSphere.00245-17.
12. **Goldstein T, Anthony SJ, Gbakima A, Bird BH, Bangura J, Tremeau-Bravard A, Belaganahali MN, Wells HL, Dhanota JK, Liang E, Grodus M, Jangra RK, DeJesus VA, Lasso G, Smith BR, Jambai A, Kamara BO, Kamara S, Bangura W, Monagin C, Shapira S, Johnson CK, Saylors K, Rubin RM, Chandran K, Lipkin WI, Mazet JAK.** 2018. The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. Nat Micro **3:**1084–1089. https://doi.org/10.1038/s41564-018-0227-2. Author correction. Nat Microbiol 2018.
13. **Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Roberston SA, editors.** 2015. Veterinary anesthesia and analgesia: The 5th edition of Lumb and Jones. Ames (IA): John Wiley and Sons. https://doi.org/10.1002/9781119421375
14. **Hall J, Rose K, Smith C, De Jong C, Phalen D, Austen J, Field H.** 2014. Health assessment of the Christmas island flying fox (*Pteropus melanotus natalis*). J Wildl Dis **50:**447–458. https://doi.org/10.7589/2013-09-245.
15. **Heard DJ, Huft VJ.** 1998. The effects of short-term physical restraint and isoflurane anesthesia on hematology and plasma biochemistry in the island flying fox (*Pteropus hypomelanus*). J Zoo Wildl Med **29:**14–17.
16. **Jacobsen KO, Villa V, Miner VL, Whitnall MH.** 2004. Effects of anesthesia and vehicle injection on circulating blood elements in C3H/HeN male mice. Contemp Top Lab Anim Sci **43:**8–12.
17. **Kading RC, Schountz T.** 2016. Flavivirus infections of bats: potential role in Zika virus ecology. Am J Trop Med Hyg **95:**993–996. https://doi.org/10.4269/ajtmh.16-0625.
18. **Machain-Williams C, López-Uribe M, Talavera-Aguilar L, Carrillo-Navarrete J, Vera-Escalante L, Puerto-Manzano F, Ulloa A, Farfán-Ale JA, Garcia-Rejon J, Blitvich BJ, Loroño-Pino MA.** 2013. Serologic evidence of flavivirus infection in bats in the Yucatan Peninsula of Mexico. J Wildl Dis **49:**684–689. https://doi.org/10.7589/2012-12-318.
19. **Malmlov A, Seetahal J, Carrington C, Ramkisson V, Foster J, Miazgowicz KL, Quackenbush S, Rovnak J, Negrete O, Munster V, Schountz T.** 2017. Serological evidence of arenavirus circulation among fruit bats in Trinidad. PLoS One **12:**1–11. https://doi.org/10.1371/journal.pone.0185308.
20. **Marini RP, Jackson LR, Esteves MI, Andrutis KA, Goslant CM, Fox JG.** 1994. Effect of isoflurane on hematologic variables in ferrets. J Vet Res **55:**1479–1483.
21. **McLaughlin AB, Epstein JH, Prakash V, Smith CS, Daszak P, Field HE, Cunningham AA.** 2007. Plasma biochemistry and hematologic values for wild-caught flying foxes (*Pteropus giganteus*) in India. J Zoo Wildl Med **38:**446–452. https://doi.org/10.1638/1042-7260(2007)38[446:PBAHVF]2.0.CO;2.
22. **McMichael L, Edson D, McLaughlin A, Mayer D, Kopp S, Meers J, Field H.** 2015. Haematology and plasma biochemistry of wild black flying-foxes, (*Pteropus alecto*) in Queensland, Australia. PLoS One **10:**1–13. https://doi.org/10.1371/journal.pone.0125741.
23. **Miller MR, McMinn RJ, Misra V, Schountz T, Müller MA, Kurth A, Munster VJ.** 2016. Broad and temperature independent Replication potential of filoviruses on cells derived from old and new world bat species. J Infect Dis **214 suppl 3:**S297–S302. https://doi.org/10.1093/infdis/jiw199.
24. **Munster VJ, Adney DR, van Doremalen N, Brown VR, Miazgowicz KL, Milne-Price S, Bushmaker T, Rosenke R, Scott D, Hawkinson A, de Wit E, Schountz T, Bowen RA.** 2016. Replication and shedding of MERS-CoV in Jamaican fruit bats (*Artibeus jamaicensis*). Sci Rep **6:**1–10. https://doi.org/10.1038/srep21878.
25. **Schinnerl M, Aydinonat D, Schwarzenberger F, Voigt CC.** 2011. Hematological survey of common neotropical bat species from Costa Rica. J Zoo Wildl Med **42:**382–391. https://doi.org/10.1638/2010-0060.1.
26. **Selig M, Lewandowski A, Kent MS.** 2016. Establishment of reference intervals for hematology and biochemistry analytes in a captive colony of straw-colored fruit bats (*Eidolon helvum*). J Zoo Wildl Med **47:**106–112. https://doi.org/10.1638/2015-0040.1.
27. **Simmons NB.** 2005. An Eocene big bang for bats. Science **307:**527–528. https://doi.org/10.1126/science.1108871.

28. **Teeling EC, Springer MS, Madsen O, Bates P, O'Brien SJ, Murphy WJ.** 2005. A molecular phylogeny for bats illuminates biogeography and the fossil record. Science **307:**580–584. https://doi.org/10.1126/science.1105113.
29. **Tomihari M, Nishihara A, Shimada T, Yanagawa M, Miyoshi M, Miyahara K, Oishi A.** 2015. A comparison of the immunological effects of propofol and isoflurane for maintenance of anesthesia in healthy dogs. J Vet Med Sci **77:**1227–1233. https://doi.org/10.1292/jvms.14-0611.
30. **Tsagkogeorga G, Parker J, Stupka E, Cotton JA, Rossiter SJ.** 2013. Phylogenomic analyses elucidate the evolutionary relationships of bats. Curr Biol **23:**2262–2267. https://doi.org/10.1016/j.cub.2013.09.014.
31. **US Department of the Interior.** [Internet]. 2017. 13 Awesome facts about bats. [Cited 12 December 2018]. Available at: https://www.doi.gov/blog/13-facts-about-bats
32. **Van Der Westhuyzen J.** 1987. Haematology and iron status of the Egyptian fruit bat, *Rousettus aegyptiacus.* Comp Biochem Physiol A Comp Physiol **90:**117–120.
33. **Williams WR, Johnston MS, Higgins S, Izzo AA, Kendall LV.** 2016. Blood profiles in unanesthetized and anesthetized guinea pigs (*Cavia porcellus*). Lab Anim (NY) **45:**35–41. https://doi.org/10.1038/laban.911.
34. **Wolk E, Bogdanowicz W.** 1987. Hematology of the hibernating bat: *Myotis daubentoni.* Comp Biochem Physiol A Comp Physiol **88:**637–639.