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EVALUATION OF HEMATOLOGIC VALUES IN FREE-RANGING AFRICAN BUFFALO (*SYNCERUS CAFFER*)

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ABSTRACT: As part of a large-scale disease screening program, blood samples were collected from 534 African buffalo (*Syncerus caffer*) in South Africa's Hluhluwe-iMfolozi Park in October 2005 and May 2006 to establish age- and sex-specific reference intervals for erythrogram and leukogram values. Sixty-seven of the animals were positive for bovine tuberculosis (TB), allowing for comparisons between TB-positive and TB-negative groups. Positive animals had basopenia and slight lymphopenia compared to TB-negative animals. Blood values were compared to those reported for captive African buffalo, American bison (*Bos bison*), and cattle (*Bos taurus*). The free-ranging buffalo sampled in this study had higher white blood cell counts than captive buffalo, and this difference was driven by lymphocytes. Free-ranging buffalo also had higher red blood cell counts, mean corpuscular hemoglobin concentration (MCHC), white blood cell counts, neutrophils and lymphocytes, and lower mean corpuscular volume (MCV) than cattle. Demographic and environmental factors strongly affected hematologic values in the study population. Older animals had significantly higher hemoglobin, hematocrit, MCV, and mean corpuscular hemoglobin (MCH), while younger animals had a higher red blood cell count, red cell distribution width (RDW), and white blood cell count, which was due to lymphocytes and basophils. Females had a higher hemoglobin concentration, hematocrit, MCV, MCH, and basophils than males. At the end of the wet season, hemoglobin, red blood cell count, hematocrit, MCHC, RDW, white blood cell count, and neutrophils were all significantly higher, while basophils and MCV were lower, than at the end of the dry season. Our results emphasize the need to use species-specific data when interpreting hematologic values and point to important differences in hematology between captive and free-ranging animals of the same species. Strong variability in hematologic values with animal age and sex, season, and herd affiliation indicates that "normal" hematologic values in wild animals vary throughout their lives and subject to fluctuating environmental conditions.

Key words: African buffalo, bovine tuberculosis, demographic variation, hematologic reference ranges, *Mycobacterium bovis*, seasonal variation, *Syncerus caffer*, tuberculosis.

INTRODUCTION

Hematologic values are a representation of the health status of the animal and, as a group, can be used to help evaluate the health of a herd. Accurate reference data are therefore essential for assessing population health, but published data on hematologic values in free-ranging wildlife are scarce. The reference intervals that are currently used for African buffalo (*Syncerus caffer*) are based upon data collected from captive buffalo or cattle, and it is unclear whether these reference ranges represent an appropriate baseline for health evaluation of free-ranging buffalo. Further, hematologic reports from wildlife species can be difficult to interpret, because typically very little hematologic information from wildlife populations with common infectious diseases is available.

As part of a large-scale disease screening program, we collected blood samples from more than 500 African buffalo in South Africa's Hluhluwe-iMfolozi Park (HIP). The buffalo were tested for bovine tuberculosis (TB), presenting an opportunity to compare hematologic values in TB-positive and TB-negative animals. Moreover, the unusually large number of animals sampled in this study allowed for assessment of variation in hematology with important demographic and environmental factors, such as sex, age, season, and herd affiliation.

Tuberculosis in bovids is a chronic debilitating disease caused by *Mycobacterium bovis* (Isaza, 2003). *Mycobacterium*

bovis has a broad host range, causing disease in cattle, bison, buffalo, and other ungulates, as well as many of their predators (Morris et al., 1994). Tuberculosis in African buffalo is an economically and socially important disease: African buffalo can serve as reservoirs for *M. bovis* (DeVos et al., 2001) and a source of infection for local cattle, an important food source and measure of wealth in southern Africa. Tuberculosis has been present in HIP since at least 1986 (Jolles et al., 2005) and is emerging as a wildlife disease throughout sub-Saharan Africa (Michel et al., 2006). The disease affects both survival and fecundity in buffalo but does not appear to cause sudden drastic population reductions (Jolles et al., 2005, 2006). Studies in cattle have shown that some animals infected with *M. bovis* have hematologic changes typical of chronic mycobacterial infection, such as leukopenia and anemia (Smith, 2001). By contrast, bison infected with *M. bovis* showed a slight increase in the number of monocytes and lymphocytes when compared to uninfected bison, but were still within the normal reference intervals determined for bison (Miller et al., 1989). To date, there is no information on hematologic responses to TB for African buffalo.

Buffalo nutrition, body condition, and the prevalence and severity of microparasitic and macroparasitic infections vary with sex, age, and environmental factors such as season and herd affiliation (Prins, 1996; Rodwell et al., 2001; Caron et al., 2003; Jolles et al., 2005; Cross and Getz, 2006; Ezenwa and Jolles, 2008; Jolles et al., 2008; Ezenwa et al., in press). Insofar as hematologic values reflect animal health, nutrition, and body condition, we would also expect hematology to vary with these demographic and environmental factors. Indeed, hematologic studies in other species have shown that younger ruminants often have higher hematocrit and increased mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) compared to older animals (Pospisil, 1985; Perez et al., 2003; Brun-Hansen, 2006). It has also been noted that males often have higher hematocrit than females (Lumsden, 1980; Perez et al., 2003), and seasonal variability in hematologic values has been demonstrated in other species such as tortoises (Lawrence, 1987) and dolphins (Hall et al., 2007). Nutrition has also been shown to affect hematologic values in water buffalo in Indonesia (Thahar et al., 1983), where animals fed a high concentrate diet had higher packed cell volumes and hemoglobin concentrations.

Here we present data from 467 clinically healthy (TB-negative) buffalo sampled at HIP to establish hematologic reference intervals for buffalo based on a large sample of free-ranging animals. The large number of animals sampled in this study allowed us to assess how demographic and environmental factors related to hematologic values and to calculate reference ranges for different age-sex groups of buffalo. Finally, we examined whether TB infection (observed in 67 buffalo) influenced the hematology of this species.

MATERIALS AND METHODS

Study system

We collected data on African buffalo captured as part of the bovine TB control program at HIP, South Africa (28°10′–28°14′S, 31°54′–32°03′N). Hluhluwe-iMfolozi Park comprises almost 900 km^2, with a buffalo population of approximately 3,000 individuals. Rainfall occurs seasonally (October through April) and on a north-south gradient (Jolles et al., 2006). Animals were captured in the Masinda section of the park over a 2-wk period in October 2005 and May 2006. Captures were carried out by KwaZulu-Natal Wildlife, the park management organization, using a helicopter and funnel system to drive buffalo herds into a capture corral. Once corralled, buffalo were anesthetized for bovine TB testing; age and sex were recorded, and blood samples were collected for hematologic analysis. In juveniles up to 2 yr (no permanent incisors), we estimated age according to body

size and horn development. In animals 2–5 yr old, we determined age from incisor emergence patterns (Grimsdell, 1973), and for buffalo aged 6+ yr we used tooth wear of incisor one to estimate age (Jolles, 2007). All captured animals were marked with brands to allow for future identification at recapture. To avoid pseudoreplication, we eliminated all recaptured individuals from the May 2006 dataset. The final dataset of 534 individuals included 467 TB-negative and 67 TB-positive animals.

Blood collection and analysis

Blood was collected via jugular venipuncture into 10 ml EDTA vacutainer tubes. Samples were immediately placed on ice and shipped to the laboratory of Dr. Bouwer & Partners Inc. (Durban, South Africa) for hematologic analysis on an ADVIA 120 automated analyzer (Bayer Diagnostics, Tarrytown, New York, USA). The values obtained included hemoglobin, hematocrit (HCT), red blood cell (RBC) count, MCV, MCH, MCHC, and red cell distribution width (RDW). The total white blood cell (WBC) count was also reported, along with differentials for neutrophils, lymphocytes, eosinophils, monocytes, and basophils.

Statistical analyses

Hematologic results were tabulated and analyzed using Analyze-It, a Microsoft Excel add-on, to determine reference intervals. The reference intervals were calculated on all TB-negative animals over the age of 1 yr after exclusion of any outliers. Outliers were identified on scatter plots and box plots. Reference intervals for normally distributed data were calculated based on the mean ± 1.96 multiplied by standard deviation. Basophils and eosinophils were nonnormally distributed, so the median was reported and a nonparametric ranking method (Linnet, 2000) was used to determine the reference intervals for basophils and eosinophils. The data were ranked in ascending order, and reference intervals were determined based on the data between the 2.5 and 97.5 percentile.

To assess differences in hematologic values related to gender and age, we used a general linear model (GLM). This approach allowed us to test for effects of categorical predictor variables (such as sex, season, herd affiliation, or TB status), as well as continuous predictor variables (such as age; McCullagh and Nelder, 1989; Crawley, 2005). It also provided a means to evaluate effects of age and sex, while controlling for herd affiliation (potential variation in habitat, diet, and activity level among herds) and for season (potential effects associated with variation in nutrition and parasite load). The strength of this approach is that it minimizes confounding and maximizes statistical power compared to splitting the sample for multiple comparisons; it allows all the available information on all individuals to be evaluated simultaneously. Our statistical model included categorical variables sex, herd, and season, along with the continuous variable age as independent effects on each hematologic (dependent) variable. Finally, to test whether hematologic values differed between TB-positive and TB-negative buffalo, we added "TB status" as an additional categorical value to the GLM. We thus controlled for the effects of age, sex, herd affiliation, and season when testing for differences between infected and healthy animals. This is important because TB prevalence differs across age groups and herds (Jolles et al., 2005).

We compare our results for free-ranging African buffalo with published hematologic data from captive African buffalo (Pospisil et al., 1985; International Species Information System [ISIS], 2007), American bison (*Bos bison*; Miller et al., 1989), and cattle (*Bos taurus*; Smith, 2001). The ISIS maintains an electronic central database of animals held in zoological institutions, and 676 member institutions provide health and genetic data on animals, with the primary objective of managing zoo populations for species conservation.

RESULTS

The reference intervals and means for the 420 TB-negative buffalo over the age of 1 yr are reported in Table 1 and compared to published values for captive African buffalo, cattle, and bison. The free-ranging buffalo tested in this study had higher WBC counts than those recorded for captive buffalo by both Pospisil (1985) and ISIS (2007). The average values for MCHC, WBC counts, and neutrophils for our sample of buffalo fall above the reference ranges published for cattle, while the buffalo average for MCV falls below the published cattle range. Smith's (2001) reference ranges for captive bison ($n=40$–89 depending on variable) are rather broad, and all of our average buffalo values fall within these ranges. The standard deviations for the

TABLE 1. Comparison of reported hematologic values for captive and free-ranging buffalo, bison, and cattle. Columns give reference ranges, and means and standard deviations of hematologic values, except for eosinophils and basophils, appear in brackets where medians are given. For cattle, only reference ranges were available.

Hematologic value[a]	Captive African buffalo (ISIS, 2007) n listed below	Captive African buffalo (Pospisil, 1985) $n=13$	Free-ranging African buffalo (this study) $n=420$	Cattle (*Bos taurus*) (Smith, 2001)	Captive bison (*Bos bison*) (Miller et al., 1989) $n=106$
Hgb (g/dl)	9–21 (13.1±2.5; $n=47$)	13–18.2 (15.4±1.9)	10.5–16.8 (13.6±1.6)	8–15	10.3–23 (18.5±0.9)
RBC (cells/μl×10^6)	5.35–15 (9.26±2.12; $n=41$)	6.72–12.10 (9.76±1.72)	7.0–12.9 (10.0±1.5)	5–10	
Hct (%)	25–67 (38.3±9.2; $n=47$)	32–47 (39.1±0.044)	28–43 (36±4)	24–46	28–59 (50.8±2.8)
MCV (μm^3)	24.5–81.5 (41.8±9.4; $n=41$)		27.8–44.4 (36.0±4.3)	40–60	
MCH	8.5–25.2 (14.3±2.7; $n=41$)		10.7–16.9 (13.8±1.6)	11–17	
MCHC (g/dl)	28.6–46.4 (34.6±3.4; $n=47$)		35.6–40.9 (38.3±1.4)	30–36	
RDW			14.4–22.2 (18.3±2.0)		
WBC (cells/μl×10^6)	2.54–13.3 (6.31±2.17; $n=45$)	5.1–12.4 (7.39±2.07)	5.8–21.1 (13.4±3.9)	4–12	3.4–25.3 (7.84±1.6)
Neutrophils (cells/μl×10^6)	0.227–6.6 (2.8±1.9; $n=34$)		1.6–9.7 (5.7±2.1)	0.6–4	0.84–12 (3.4)
Lymphocytes (cells/μl×10^6)	0.813–8.9 (2.56±1.6; $n=34$)		1.5–12.8 (7.1±2.9)	2.5–7.5	1.5–11 (3.9)
Monocytes (cells/μl×10^6)	0.032–1.63 (0.186±0.334; $n=24$)		0–1.1 (0.28±0.41)		0–1.3 (0.24)
Eosinophils (cells/μl×10^6)	0.054–0.68 (0.25±0.17; $n=22$)		0–0.7 median=0.2		0–1.5 (0.171)
Basophils (cells/μl×10^6)	0.025–0.284 (0.093±0.073; $n=14$)		0–0.3 median=0.1		0–0.3 (0.021)

[a] Hct = hematocrit, Hgb = hemoglobin, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, RBC = red blood cell count, RDW = red cell distribution width, WBC = white blood cell count.

erythrogram data calculated from our larger sample size of buffalo were smaller than those reported in ISIS, yielding tighter reference intervals for African buffalo; but leukogram data were much more variable in our study population.

The effects of demographic and environmental variables on hematology in buffalo are reported in Table 2. Both age and sex affected hematologic values. Older animals showed significantly higher hemoglobin, hematocrit, MCV, and MCH values. Conversely, they had significantly lower counts for total WBC, driven by lower basophil and lymphocyte counts, and lower total RBC counts and RDW. The erythrogram data for hemoglobin, hematocrit, MCV, MCH, and the leukogram data for basophils were all significantly higher in females than males. Since age and sex both had significant effects on hematologic values, we recalculated reference intervals for specific age and sex groups in our sample of buffalo (Table 3).

Season had a significant effect on many of the erythrogram and leukogram values. Hemoglobin, RBC counts, hematocrit, MCHC, RDW, WBC counts, and neutrophils were all higher in May, at the end of the wet season, compared to the end of the dry season in October. Basophils and MCV were significantly higher in October than in May. Herd affiliation affected all hematologic values except MCH and eosinophils.

TB-negative animals had significantly higher basophil counts and marginally higher lymphocyte counts than TB-positive individuals, but no other hematologic values were influenced by TB status. Though statistically significant, these differences are slight and not very helpful in terms of diagnosing TB, because basophils and lymphocytes for TB-positive buffalo fell within the reference range for healthy animals of their age and sex.

DISCUSSION

The sample size obtained in this study was much larger than other reported studies for African buffalo, yielding tighter reference intervals and smaller standard deviations for erythrogram data. Our study animals were also of similar background in that they were all free-ranging buffalo from the same population, which may have further reduced the variability in erythrogram results, compared to previous studies using captive buffalo from a variety of backgrounds. By contrast, leukogram profiles were very variable in this study, giving much broader reference ranges than those reported to date. On average the WBC counts in our buffalo population were higher than in either captive buffalo or cattle. Wild animals are continually being challenged immunologically by a variety of infectious agents (Fowler and Miller, 2007), and these challenges may be much greater and more variable than immunologic challenges to captive or domestic animals kept in controlled facilities, resulting in greater investment in immunity and therefore higher WBC counts. Another possible explanation is stress-induced lymphocytosis causing the leukocytosis. Stress causes epinephrine release and lymphocytosis in cattle (Smith, 2001).

Many other hematologic values in our sample of buffalo differed from published reference ranges for cattle and captive buffalo. The studies certainly used different methods and equipment to analyze the blood so some variation is expected. Reference intervals are usually derived from populations of clinically healthy animals. We did exclude TB-positive animals from our reference range determination, but health status was not otherwise evaluated. This may have influenced our reference ranges, especially the wide variability of the leukograms. Two of the comparison studies had sample sizes of less than 40 (Pospisil, 1985; ISIS, 2007), so that minimum-maximum ranges, rather than reference ranges, were shown. Despite these difficulties, the differences in hematology between cattle and captive and free-ranging buffalo emphasize the importance of using species-specific ref-

TABLE 2. Effect of age, sex, season, herd affiliation, and tuberculosis (TB) status on hematology in African buffalo. Only animals older than 12 mos were included. A positive coefficient β for the effect of age indicates that older animals have higher hematologic values. A positive coefficient β for the effect of sex indicates that the hematologic value is greater in females than in males. A positive coefficient β for the effect of TB status indicates that TB-negative animals have higher hematologic values than TB-positive animals. A positive coefficient β for the effect of season indicates that hematologic values were higher in October than in May. Statistically significant ($P=0.05$) results are designated with an asterisk (*), marginally significant ($0.05 \leq P \leq 0.1$) results are marked with a plus ($^+$).

	Age			Sex			Season			Herd		TB status		
Hematologic value[a]	F[b]	P	β	F	P	β	F	P	β	F	P	F	P	β
Hgb (g/dl)	8.0	0.00497*	0.04	8.1	0.00467*	0.17	48.4	0.00000*	−0.79	8.7	0.00000*	0.0	0.98828	0.00
RBC (cells/μl×10^6)	118.7	0.00000*	−0.13	0.6	0.44619	−0.04	40.4	0.00000*	−0.63	8.2	0.00000*	2.0	0.15360	0.11
Hct (%)	9.0	0.00285*	0.001	6.4	0.01197*	0.004	25.4	0.00000*	−0.02	8.3	0.00000*	0.0	0.87331	−0.00
MCV (μm^3)	363.4	0.00000*	0.60	19.2	0.00001*	0.62	9.0	0.00278*	0.80	5.7	0.00000*	2.5	0.11801	−0.31
MCH	365.5	0.00000*	0.23	23.7	0.00000*	0.26	0.9	0.33560	0.10	2.0	0.05820$^+$	2.3	0.12718	−0.11
MCHC (g/dl)	1.9	0.17269	−0.01	2.9	0.08687$^+$	0.08	41.8	0.00000*	−0.58	25.9	0.00000*	0.0	0.88909	−0.01
RDW	17.6	0.00003*	−0.06	0.1	0.72636	0.02	57.2	0.00000*	−0.97	18.5	0.00000*	0.8	0.35932	0.09
WBC (cells/μl×10^6)	104.2	0.00000*	−0.36	2.3	0.13105	0.24	25.9	0.00000*	−1.51	9.5	0.00000*	2.9	0.08902$^+$	0.38
Neutrophils (cells/μl×10^6)	0.1	0.73413	0.01	0.1	0.74274	0.03	43.7	0.00000*	−1.15	9.9	0.00000*	0.2	0.66378	0.06
Lymphocytes (cells/μl×10^6)	209.1	0.00000*	−0.35	2.8	0.09234$^+$	0.19	2.7	0.10324	−0.34	6.5	0.00000*	3.7	0.05571$^+$	0.30
Monocytes (cells/μl×10^6)	0.1	0.72686	−0.00	1.2	0.27744	−0.02	0.0	0.92601	0.00	19.1	0.00000*	0.7	0.40971	0.02
Eosinophils (cells/μl×10^6)	1.4	0.23387	−0.02	1.3	0.24925	0.08	0.1	0.75473	−0.04	0.4	0.88183	0.0	0.90567	0.01
Basophils (cells/μl×10^6)	67.5	0.00000*	−0.004	16.0	0.00007*	0.01	6.9	0.00908*	0.01	41.9	0.00000*	7.9	0.00526*	0.01

[a] Hgb = hemoglobin, RBC = red blood cell count, Hct = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width, WBC = white blood cell count.

[b] F is the test statistic from which significant levels (P values) for general linear models are calculated.

TABLE 3. Reference intervals in the various age and sex groups after excluding all TB+ buffalo. Because of smaller sample size buffalo under one year of age had to be combined into one group irrespective of sex. All other age groups were separated into male and female ranges. There are also overall ranges listed, as well as ranges for all females and all males irrespective of age. Reference intervals are listed first, followed by mean ± standard deviation in parentheses. Eosinophils and basophils were not normally distributed, so the median is reported instead of the mean.

Hematologic value[a]	All ≥1 yr n=420	Female ≥1 yr n=247	Male ≥1 yr n=173	All <1 yr n=47	Male 1–3 yr n=125	Female 1–3 yr n=123	Male ≥4 yr n=48	Female ≥4 yr n=124
Hgb (g/dl)	10.5–16.8 (13.6±1.6)	10.9–16.72 (13.8±1.5)	10.0–16.7 (13.4±1.7)	8.5–13.9 (11.2±1.4)	10.1–15.9 (13 0±1.5)	10.6–16.9 (13.8±1.6)	10.4–18.1 (14.2±1.9)	11.2–16.5 (13.9±1.4)
RBC (cells/μl×106)	7.0–12.9 (10.0±1.5)	6.9–12.75 (9.9±1.5)	7.2–13.1 (10.2±1.5)	6.9–14.0 (10.4±1.8)	7.3–13.2 (10.2±1.5)	7.8–13.3 (10.6±1.4)	7.1–13.2 (10.2±1.6)	6.8–11.4 (9.1±1.2)
Hct (%)	28–43 (36±0.04)	29–43 (36±4)	27–43 (35±4)	23–36 (30±3)	27–41 (34±4)	28–44 (36±4)	28–46 (37±5)	30–43 (36±3)
MCV (μm^3)	27.8–44.4 (36.0±4.3)	28.5–45.8 (37.2±4.4)	27.7–41.2 (34.4±3.5)	22.3–35.6 (29.0±3.4)	27.2–39.8 (33.5±3.2)	27.8–40.7 (34.2±3.3)	31.2–42.5 (36.9±2.9)	33.5–46.6 (40.1±3.4)
MCH	10.7–16.9 (13.8±1.6)	11–17.5 (14.2±1.7)	10.9–15.5 (13.2±1.2)	8.8–13.0 (10.9±1.1)	10.8–14.9 (12.8±1.1)	10.8–15.4 (13.1±1.2)	11.9–16.2 (14.1±1.1)	13.0–17.8 (15.4±1.2)
MCHC (g/dl)	35.6–40.9 (38.3±1.4)	36.1–40.5 (38.3±1.1)	35.0–41.4 (38.2±1.6)	34.7–40.1 (37.8±1.6)	34.7–41.6 (38.1±1.8)	35.9–40.8 (38.3±1.3)	35.6–40.7 (38.3±1.3)	36.3–40.3 (38.3±1.3)
RDW	14.4–22.2 (18.3±2.0)	14.6–21.9 (18.3±1.9)	14.2–22.6 (18.4±2.1)	14.6–25.2 (19.9±2.7)	14.1–22.7 (18.4±2.2)	14.4–22.7 (18.6±2.1)	14.5–22.5 (18.5±2.0)	14.9–21.0 (17.9±1.5)
WBC (cells/μl×106)	5.8–21.1 (13.4±3.9)	5.6–21.0 (13.3±3.9)	6.1–21.2 (13.6±3.9)	6.7–23.0 (14.8±4.2)	6.3–22 (14.1±1.4)	7.0–22.9 (14.9±4.1)	6.3–18.2 (12.2±3.0)	5.8–17.7 (11.7±3.1)
Neutrophils (cells/μl×106)	1.6–9.7 (5.7±2.1)	1.8–9.6 (5.7±2.0)	1.2–9.9 (5.6±2.2)	0.4–8.4 (4.4±2.0)	1–9.9 (5.4±2.3)	2.0–9.6 (5.8±1.9)	2.3–9.9 (6.1±2.0)	1.7–9.6 (5.7±2.0)
Lymphocytes (cells/μl×106)	1.5–12.8 (7.1±2.9)	1.2–12.6 (6.9±2.9)	1.9–12.9 (7.4±2.8)	4.6–14.7 (9.6±2.6)	2.8–13.4 (8.1±2.7)	2.7–14.1 (8.4±2.9)	1.4–9.7 (5.5±2.1)	1.5–9.5 (5.7±2.0)
Monocytes (cells/μl×106)	0–1.1 (0.3±0.4)	0–1 (0.3±0.3)	0–1.2 (0.3±0.5)	0–1.3 (0.4±0.4)	0–0.8 (0.3±0.3)	0–0.8 (0.3±0.3)	0–1.9 (0.3±0.8)	0–1 (0.3±0.4)
Eosinophils (cells/μl×106)	0–0.7 median=0.2	0–0.8 median=0.2	0–0.6 median=0.2	0–1 median=0.2	0–0.6 median=0.2	0–0.7 median=0.3	0–1 median=0.3	0–0.7 median=0.2
Basophils (cells/μl×106)	0–0.3 median=0.11	0–0.3 median=0.1	0–0.3 median=0.1	0–0.4 median=0.2	0–0.3 median=0.1	0–0.3 median=0.1	0–0.2 median=0.1	0–0.3 median=0.1

[a] Hct = hematocrit, Hgb = hemoglobin, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, RBC = red blood cell count, RDW = red cell distribution width, WBC = white blood cell count.

erence intervals from natural populations whenever available for interpreting hematology results in wild animals.

Age and sex strongly affected many hematologic values in buffalo. Interpreting individual hematologic results against reference ranges derived from the total population can therefore lack sensitivity, failing to flag some results that might be unusual for a particular age-sex class. For example, juvenile animals are setting up their adaptive immune response and increased lymphocytes are typically observed in younger animals due to antigen presentation and response. Accordingly, we observed higher WBC counts, driven by high lymphocyte counts, in juvenile buffalo compared to adults. We also noted higher hemoglobin and hematocrit in females than in males. Further research will investigate whether this unexpected pattern is related to female reproductive status, as many of the females we handled were lactating or pregnant.

Season had very clear effects on hematologic values in the buffalo we sampled. At the end of the wet season in May, buffalo generally had higher erythrogram values, whereas in October, following the dry season, the animals were comparatively anemic and in poorer nutritional condition, as indicated by low hematocrit, RBC counts, and hemoglobin concentrations. Buffalo at HIP experience strong seasonal variation in forage quality and availability (Kleynhans, 2005), resulting in much poorer body condition in October than in May (Jolles, unpubl. data). This seasonal pattern in nutrition and buffalo body condition most likely underlies the differences in erythrogram values reported here. Buffalo also had lower WBC counts and neutrophil levels following the dry season, and this may be due to nutritional limitations that prevent individuals from allocating scarce resources to immune protection during periods of poor resource quality. Exposure to infectious agents may also decline during the dry season, especially for environmentally transmitted parasites that are vulnerable to desiccation, reducing the need to mount strong immune responses.

Herd affiliation strongly affected most hematologic values in our study population. Buffalo herds in HIP do not range across the whole park but limit their activities to well-defined, largely nonoverlapping home ranges (Dora et al., 2004). Rainfall, soil types, and vegetation vary across the park, and this habitat variability may affect buffalo nutrition, activity patterns, and exposure and susceptibility to parasites and pathogens (Anderson, 1993; Tanner and Michel, 1999; Cunningham-Rundles et al., 2005; Smith et al., 2005). Buffalo condition and health are thus likely to vary with environmental factors associated with herd affiliation, and these differences may be reflected in the blood values we measured.

The TB-infected buffalo had significantly lower basophil counts and marginally lower lymphocyte counts than healthy individuals. However, the values were within the normal reference intervals for TB-negative buffalo presented in this study; thus hematologic examination of TB-infected individuals would fail to detect any abnormalities. Previously reported hematologic findings of TB-infected bovids have yielded mixed results. Cattle infected with *M. bovis* often show leukopenia and anemia (Smith 2001), but TB-infected bison had a slight increase in numbers of monocytes and lymphocytes when compared to uninfected bison (Miller et al., 1989). Chronically infected opossums show lymphopenia and eosinopenia (Buddle et al., 1994). Buffalo in this study showed a decrease in lymphocytes similar to infected cattle and opossums but were inconsistent with findings from the bison study. The bison study was conducted on captive animals experimentally infected with TB, while we studied free-ranging buffalo with natural infections, making observed differences in hematologic results difficult to interpret. For example, duration of infection likely dif-

fered between the two studies, because buffalo may have been infected for a longer period of time before the samples were obtained compared to the bison that were acutely infected at the time of sampling. Animals with acute infections upregulate their lymphocyte production (Miller et al., 1989), but as the infection progresses lymphocytes become sequestered in the tubercles (Bloom, 1994; Thoen et al., 2006), which may lead to a decrease in circulating lymphocytes in chronically infected animals.

The reference intervals obtained in this study will be useful for evaluating hematologic values of wild African buffalo in the future. Differences between cattle and captive and free-ranging buffalo underscore the need to use species-specific data when available and suggest that even among animals of the same species, captive and free-ranging populations can show very distinct hematologic profiles. Furthermore, our data show strong variability with animal age and sex, season, and herd affiliation, emphasizing that "normal" hematologic values in wild animals vary throughout their lives and subject to fluctuating environmental conditions.

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