Hematologic and serum chemistry reference intervals for free-ranging lions (*Panthera leo*)

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Abstract

Hematologic and serum chemistry values are used by veterinarians and wildlife researchers to assess health status and to identify abnormally high or low levels of a particular blood parameter in a target species. For free-ranging lions (*Panthera leo*), information about these values is scarce. In this study, 7 hematologic and 11 serum biochemistry values were evaluated from 485 lions from the Kruger National Park, South Africa. Significant differences between sexes and sub-adult (≤36 months) and adult (>36 months) lions were found for most of the blood parameters and separate reference intervals were made for those values. The obtained reference intervals include the means of the various blood parameters values measured in captive lions, except for alkaline phosphatase in the subadult group. These reference intervals can be utilized for free-ranging lions, and may likely also be used as reference intervals for captive lions.

Keywords: lions, *Panthera leo*, hematology, serum chemistry, reference intervals

Blood parameter values can be used by veterinarians or researchers for the assessment of the health of animals or a population. These blood parameter values may vary between free-ranging and captive species, which was shown for e.g. African buffalo (Beechler et al., 2009). Information on hematologic and blood chemistry values is available for captive lions (*Panthera leo*) (Hawkey and Hart, 1986, International Species Information System (ISIS), 2002), but is scarce for free-ranging lions. Therefore, the aim of this study was to establish reference values for free-ranging lions for commonly used hematologic and blood chemistry values, adjusted for sex and age, and to compare these with values found for captive lions.

Lion blood samples were collected from 485 free-ranging lions from 1993-2008 in the Kruger National Park, South Africa. The complete lion data set is described elsewhere (Maas et al., 2012). The selection of the lions used for the current study was based on the body condition, only including clinically healthy lions with a good body condition score (scored 4/5 and 5/5, where 5 represents an excellent body condition).

Lions were immobilized with a combination of tiletamine and zolazepam (Zoletil® 100, Virbac) and venous blood samples were obtained from the medial saphenous vein as soon as possible, but usually within 30-60 minutes after anaesthesia, in EDTA and serum Vacutainer® tubes. Blood tubes were kept at ambient temperature and were processed within preferably 8, but maximum 24 hours. Lions were aged by examining dental attrition according to Smuts et al. (Smuts et al., 1978). Hematology analysis of EDTA samples was performed in the the Kruger National Park within 12 hours with a Coulter AcT diff analyzer (Beckman Coulter). Samples for serum chemistry were frozen at -20°C until analysis. This serum chemistry analysis was conducted with a NExCT/VetEX (Bayer Health) at The Clinical Pathology Laboratory, Onderstepoort Veterinary Academic Hospital, Faculty of Veterinary Science, University of Pretoria.

The exact numbers of lions that were used for each individual blood parameter are listed in table 1 and table 2. Seven hematological parameters were measured (hemoglobin (Hgb), haematocrit (Hct), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular

Table 1. Reference interval for seven hematologic parameters of free-ranging lions.

		Free-ranging lions				ISIS/ captive lions				
Parameter	**group**	**n**	**Mean**	**SD**	**Reference interval**	**n**	**Mean**	**SD**	**Minimal value**	**Maximal value**
Hgb (g/dL)	All	324	11.8	1.47	**8.9 - 14.6**	766	13	2.0	4.9	23
Hct (%)	All	324	35.5	4.43	**26.8 - 44.1**	890	39.1	5.4	24.8	54.0
RBC ($x10^6$cells/µl)	M, ≤36	44	6.7	0.81	**5.1 – 8.3**	68	7.47	1.32	3.8	11.7
	F, ≤36	41	6.7	0.89	**5.0 – 8.4**	93	7.06	1.11	3.8	9.98
	M, >36	69	7.2	0.78	**5.7 – 8.7**	222	8.28	1.17	5.1	14.0
	F, >36	104	6.8	0.83	**5.2 – 8.5**	349	7.96	1.07	5.22	11.0
MCV (fL)	M	113	51.3	2.4	**46.6 – 55.9**	285	49.9	5.1	29.9	76.0
	F	145	52.3	2.2	**48.0 – 56.7**	441	50.0	4.1	21.4	64.0
MCH (pg/cell)	M	113	16.7	0.94	**14.8 – 18.5**	266	16.5	1.5	11.2	27.2
	F	145	17.3	0.94	**15.5 – 19.1**	432	16.6	1.4	7.2	22.0
MCHC (g/dL)	M	113	32.6	1.51	**29.6 – 35.5**	280	33.0	3.0	20.4	42.8
	F	145	33.1	1.35	**30.4 – 35.7**	469	33.2	2.9	20.5	49.7
WBC ($x10^3$cells/µl)	≤36	87	16.4	4.69	**7.2 – 25.6**	176	12.15	4.64	4.73	31.2
	>36	183	19.0	4.73	**9.7 – 28.2**	683	13.38	4.08	5.5	28.2

For the presentation of the ISIS values the same groups were evaluated as for the free-ranging lions from the study.

Table 2. Reference intervals for eleven serum chemistry parameters of free-ranging lions.

		Free-ranging lions				ISIS/ captive lions				
analyte	**group**	**n**	**Mean**	**SD**	**Reference interval**	**n**	**Mean**	**SD**	**Minimal value**	**Maximal value**
Cholesterol (mmol/L)	M, ≤36	69	3.4	0.82	**1.8 – 5.0**	56	4.6	1.14	2.33	6.63
	F, ≤36	49	3.4	1.30	**0.9 – 6.0**	88	4.9	1.17	2.36	7.64
	M, >36	104	3.0	0.77	**1.5 – 4.6**	201	3.9	0.91	1.50	6.48
	F, >36	138	3.4	0.86	**1.7 – 5.1**	343	4.6	1.17	1.43	8.78
Total protein (g/L)	All	360	85.4	9.58	**66.7 – 104.2**	750	74	7	53	97
Albumin (g/L)	All	360	28.0	NA	**20.0 - 39.0**	692	33	5	19	56
Globulin (g/L)	≤36	118	55.2	10.85	**33.9 – 76.5**	146	34	8	19	57
	>36	242	58.0	8.73	**40.9 – 75.1**	530	43	6	26	63
A:G ratio	≤36	118	0.52	NA	**0.31 - 0.89**	-	-	-	-	-
	>36	242	0.48	NA	**0.29 – 0.81**	-	-	-	-	-
Alpha glob (mg/L)	All	360	15.5	2.57	**10.5 – 20.5**	4	13*	-	-	-
Beta glob (mg/L)	All	360	10.2	NA	**5.5 - 18.8**	5	8	2	5	10
Gamma glob (g/L)	≤36	118	22.6	5.61	**11.6 – 33.6**	-	-	-	-	-
	>36	242	25.5	5.57	**14.6 – 36.4**	7	29	10	19	41
BUN (mmol/L)	All	360	17.9	7.30	**3.6 - 32.2**	811	11.4	3.21	4.28	29.27
Creatinine	≤36	118	145.2	44.29	**58.4 –**	165	159	62	53.0	327

(µmol/L)					**232.0**					
	>36	242	182	52.71	**78.7 – 285.3**	620	239	53	0	424
Alkaline phosphatase (IU/L)	≤36	118	36.2	22.88	**0 – 81.0**	163	105	61	16	355
	>36	242	12.7	8.84	**0 – 30.0**	595	19	14	0	89

For the presentation of the ISIS values the same groups were evaluated as for the free-ranging lions from the study. NA= not applicable. Since these values were log-transformed, the SD is not given, as this value cannot be log-transformed. - = not available.

* Four lions had values for both alpha 1 and alpha 2 in the ISIS database. These were expected to be the same lions, and the total alpha value is thus given; SD for these lions cannot be given for this

hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC)) and 11 serum chemistry parameters (cholesterol, total protein, albumin, globulin, albumin: globulin ratio (AG ratio), alpha globulins, beta globulins, gamma globulins, blood urea nitrogen (BUN), alkaline phosphatase).

General linear models with the blood parameter as dependent variable were used to determine the effect of explanatory values sex (binary; M or F) and age (binary; ≤36 months or >36 months). The residuals of these models were checked visually in Q-Q plots to assess normality of the blood parameter values. When residuals were not normally distributed, the variable was log-transformed and model residuals were checked again in a Q-Q plot.

When one or both of the explanatory values had a significant influence ($p<0.05$), the mean, standard deviation (SD) and reference interval (mean±1.96 SD) were calculated separately for the different groups. If not, all lions were grouped together and one mean, SD and reference interval were given. For normally distributed data the mean, SD and the reference interval are reported. For log-transformed data, the mean and the reference interval are reported in the original scale. For the statistical analyses R version 2.15 was used (R Development Core Team, 2012). Values were compared with the values for captive lions

(mean, SD, minimal and maximal value) of the International Species Information System (ISIS), which maintains an electronic database of animals held in zoological institutions and to which member institutions provide health and genetic data (International Species Information System (ISIS), 2002).

Age of the lions in the database ranged from 3 to 144 months old (≤ 36 months: 172 lions, > 36 months: 313 lions). The following parameters were normally distributed only after log-transformation: albumin, AG ratio and beta globulins. Estimated reference intervals for all hematological and serum chemistry parameters are shown in respectively table 1 and table 2. No explicit differences were found comparing the found results with the values of captive lions, and except for alkaline phosphatase (subadult group), the reference intervals all included the mean values found for captive lions. Text books about exotic animal medicine are often based on these values of captive animals (Wack, 2003). However, no information about health status is available for the ISIS values and interpretation is complicated by the fact that they may include multiple samples per animals. Furthermore, normality of these data is not reported, thus it is unclear if the mean and standard deviation can be used to establish reference intervals.

The lions used for this study were all in a clinically healthy state, residuals of the models were normally distributed (for albumin, AG ratio and beta globulins after log-transformation) and thus reference intervals could be calculated. It could therefore be argued that these reference intervals are a better representation of blood parameter values for lions than those collected from the captive lions. However, also these reference intervals have their limitations.

Part of these lions have been used in another study that showed that diseases (viz. feline immunodeficiency virus and bovine tuberculosis (BTB)) may account for significant differences in blood value parameters (Maas et al., 2012). For the lions in this study, underlying diseases were not accounted for, for several reasons. First, though information about the BTB disease status was known, as well as the status for several viral infections, nothing was known about e.g. parasitic infections. We therefore thought it was incorrect to

classify animals negative for the tested infections as “healthy”. Also, making general linear models with infectious diseases as explanatory variables would reduce the number of lions with complete datasets available drastically, thus decreasing the reliability of the reference intervals. Furthermore, as often the infection status for all these infectious diseases is unknown for individual lions, it is debatable what advantage this would have for veterinarians or researchers. Therefore, we only included lions in a clinically healthy state and disregarded by definition incomplete disease status information.

Blood samples were collected under field circumstances, and no standardized protocol was used for the transport to the laboratory. However, as most lions were captured at night in the winter season, temperatures during transport should have been within a reasonable range, and the large number of samples should compensate for this.

This dataset contained only lions from the Kruger National Park in South Africa. Lions from other locations may have somewhat different blood parameter values, but these differences are expected to be only small for clinically healthy lions.

We conclude that these reference intervals can be used by veterinarians and wildlife researchers to assess the health status of free-ranging lions, and we propose they can also be used for captive lions as they are arguably more informative than the currently available mean, SD, minimal and maximal values.

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References

Beechler, B.R., Jolles, A.E., Ezenwa, V.O., 2009. Evaluation of hematologic values in free-ranging African buffalo (Syncerus caffer). Journal of Wildlife Diseases 45, 57-66.

Hawkey, C.M., Hart, M.G., 1986. Haematological reference values for adult pumas, lions, tigers, leopards, jaguars and cheetahs. Research in Veterinary Science 41, 268-269.

International Species Information System (ISIS), 2002. Physiological Data Reference Values. CD-ROM, Teare, J.A. (Ed.), Apple Valley, USA.

Maas, M., Keet, D.F., Rutten, V.P.M.G., Heesterbeek, J.A.P., Nielen, M., 2012. Assessing the impact of feline immunodeficiency virus and bovine tuberculosis co-infection in African lions. Proceedings of the Royal Society B: Biological Sciences 279, 4206-4214.

Smuts, G.L., Anderson, J.L., Austin, J.C., 1978. Age determination of the African Lion (Panthera leo). Journal of Zoology, London 185, 115-146.

R Development Core Team, 2012. R: A Language and Environment for Statistical Computing version 2.15.0.

Wack, R.F., 2003. Felidae. In: Fowler, M.E., Miller, R.E. (Eds.), Zoo and Wild Animal Medicine, Saunders, Missouri, pp. 491-501.