Health monitoring of African leopard (*Panthera pardus pardus*): ratio of WBC to RBC of captive vs. wild leopards.

By

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HEALTH MONITORING OF AFRICAN LEOPARD (*PANTHERA PARDUS PARDUS*): USING RATIOS OF WBC TO RBC OF CAPTIVE VS. WILD LEOPARDS

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Leopards are one of the big cats belonging to the genus *Panthera*. They, according to Bothman & Walker (2013) occupy more diverse habitats than any other mammal except man and other rodents. In southern Africa, leopards are the most common large predator (Bothman & Walker 2013), inhabiting regions which vary from open and arid savannas or desert to forests and coastal mountain. Those inhabiting savannah are bigger than those found in deserts and mountains (Stuart & Stuart 2011, Apps 2012, SANBI 2020). Although leopards found in South Africa have different names, for instance, the Cape leopard and the Kalahari leopard, they are no differences between the two except for the region inhabited.

Internationally, various studies (See table 1) have been conducted on the haematology of various big cats belonging to the genus *Panthera*. Generally, the aims were to provide information on hematological values for diagnostic and physiological status purposes for captive big cats. The reason for such studies to be conducted is due to the dwindling of populations of species belonging to genus *Panthera* and also to urge governments to develop more protected areas for the sustenance of such cats (Balme et al. 2009, Swanepoel et al. 2014, Jacobson et al. 2016, and Swanepoel et al. 2016). African leopard (*Panthera pardus pardus*), specifically, are widely distributed in Southern Africa, but now absent from the sheep farming areas of central South Africa (Stuart et al. 2007).Thus the development of protected areas throughout Southern Africa in order to conserve them. However, the presence of Protected Areas (PAs) still does not stop mortality (Balme & Hunter 2004).

According to Swanepoel et al. (2014) however, the African leopard's mortality rates are higher in wild compared to captive areas. Such results were not obtained from haematological values of leopards inhabiting both protected and non-protected areas, but from external factors causing mortality such as trophy hunting and conflict with farmers. Captive animals live longer and are much healthier that wild animals mainly because of the current contributions of veterinarians in preventing/treating diseases that threaten species' survival (Deem 2008). Using haematological values of leopards, I will compare the ratio of WBC to RBC of captive leopards against wild leopards in order to determine whether captive leopards are healthier than wild leopards.

Leopards are genetically diverse and widely distributed globally (Uphyrkina et al. 2001). Although there is a large variety of African leopard, the Cape and Kalahari leopard is the most common African leopard found in South Africa. These leopards are considered to be one of the big 5 tourist attraction, and those that hold traditional meanings to certain local cultures as their skin is used for clothing during traditional customs. Moreover, the leopard is also one of a few animals occurring in South African money note, the R200 note. Thus there is a need for such a species to be protected. It is not only due to their conservation status, but economic and traditional benefits (Lockey 2017, Ajayi 2019) the species provides for our country.

All the mentioned studies (See table 2) were not comparing the ratios of WBC to RBC of captive leopards against those of wild leopards. Their results, taking Singh et al. (1999) as an example showcased the values of, for example, Total Erythrocyte count (TEC), Packed cell volume (PCV),Blood Clotting Time (BCT) and Hemeglobin (hb) of leopards and other species belonging to genus *Panthera*. All these values specifically that of TEC, and TLC (Total Leukocyte Count) from Sabapara et al., (2008) and Salakij et al (2009) were, firstly found to be similar in leopards, tigers, tigeress and clouded leopards (Singh et al. 1999), secondly, the mean TEC of leopards from Sabapara et al., (2008) was found to be comparable to that of Singh (1999), Wallach & Boever (1983). Thirdly, the TLC value found in Sabapara et al. (2008) was comparable to that of Hawkey & Hart (1986).

Since these studies did not take a ratio perspective in analysing raw values, showing a gap in these types of research on big cats, I will be using ratios of WBC to RBC to determine which leopards (captive or wild) are healthier. Given previous research's results, I think that the ratio of WBC to RBC of protected leopards will not be equivalent to the ratio of WBC to RBC of non protected leopards because protected areas have been recently shown to benefit a wide range of groups such as plants, mammals, birds and insects by reducing mortality rates as animals will not easily be prone to diseases and will be healthier (Gray et al. 2016).

Table 1: Aims and objectives of previous studies on haematological values of big cats, *****: Studies focusing on leopards (*Panthera pardus*) haematological values (See table 2 for results of studies in terms of RBC and WBC count.)

Authors	Focus of study	Aims and objectives of study
Singh et al. 1999*	Haematology of tigers (*Panthera tigris tigris*), leopards (*Panthera pardus*) and clouded leopards (*Neofelis nebulosa)*	To have a base line data to provide basic information on normal haematological values in tropical climate for identifying abnormalities in blood of sick tigers (*Panthera tigris*), leopards (*Panthera pardus*), and clouded leopards (*Neofelis nebulosa*) kept under captivity in tropical region.
Sabapara et al. 2008*	Haematological reference intervals for Indian Leopards (*Panthera pardus*)	To have a base line information on haematological parameters as a reference value of Indian leopards (*P.p fusca*)
Erasmus, 2009	Determination of some blood parameters in the African lion (*Panthera* leo)	To have some blood parameters of the African lion (*Panthera* leo)
Salakij et al. 2009*	Haematology, cytochemical and ultrastructural characteristics of blood cells in leopard (*Panthera pardus*)	To provide a guide for the haematology, identification of the morphology, cytochemistry and ultrastructure of blood cells in leopards that is useful for zoological veterinarians in leopard conservation
Mussart et al. 2009	Haematological variables of healthy captive “yaguareté” (*Panthera onca*)	To obtain normal values for the main blood parameters with diagnostic utility in healthy specimens of *P. onca* from a Northeast Argentina reserve.
Shrivatav et al. 2012	Haematological and biochemical studies in tigers (*Panthera tigris tigris*)	To establish haematological and biochemical parameters for health monitoring of Bengal tigers (*Panthera tigris tigris*) in free range habitats.
Hussain et al. 2016	Haematological and blood chemistry values in snow leopard (*Panthera uncia*)	To determine the basic haematological and blood chemistry of snow leopards (*Panthera uncia*)

Table 2: RBC and WBC count of leopards (*Panthera pardus*) from previous research (±SD.)

Authors	Female leopards		Male leopards		Overall	
	RBC count	WBC count	RBC count	WBC count	RBC count	WBC count
Singh et al. 1999	6.96±0.16					
	(6.48-7.67)					
Salakij et al. 2009	7.280±1.071	11.929±3.287	8.739±1.187	15.008±3.357	7.795±1.293	13.016±3.546
Sabapara et al. 2008					7.06 ± 0.19 x106/cmm	13100±327/cmm

Material and methods

The donated samples of nine protected leopards and eight non-protected leopards inhabiting different locations in South Africa were included for this study between January 2013 and December 2015 (See table 3).The study was neither age nor sex biased as study subjects from both environments were both male and female and there were no age restrictions during data collection. Although the focus was on blood sample collection, certain aspects were also considered as they have an effect on the health of study subjects. For instance, physical condition, diet, parasite treatment and weight of leopards.

Table 3: Study subjects sampled between January 2013 and December 2015.

Captive Leopards				**Wild Leopards**			
Study subjects	**Sex**	**Age**	**Location**	**Study subjects**	**Sex**	**Age**	**Location**
Cap L 1	Female	Adult	BFNZoo	*WL 1*	Female	Adult	LA
Cap L 2	Female	Adult	CHXP	*WL 2*	Female	Adult	LA
Cap L 3	Female	Sub adult	*CHXP*	*WL 3*	Female	Sub adult	GKCA
Cap L 4	Female	Adult	*CHXP*	*WL 4*	Male	Adult	LRC
Cap L 5	Female	Adult	MOH	*WL 5*	Male	Adult	LRC
Cap L 6	Male	Sub adult	CHXP				
Cap L 7	Male	Adult	CHXP				
Cap L 8	Male	Adult	CHXP				
Cap L 9	Male	Sub adult	MOH				

BFNZoo-Bloemfontein Zoo (Free State)

CHXP- Cheetah experience (Free State)

GKCA- Greater Kruger Conservation Area. (Limpopo)

LA- Lydenburg surrounding area (Mpumalanga)

LRC-Lajuma Research Centre

MOH-Moholoholo Wildlife Rehabilitation Centre

Captive leopards sampling

These leopards (Cap L) were sedated and sampled at two facilities in the Free State and Mpumalanga province by a qualified veterinarian following standards and procedures as stated by the National Registered Ethics Animal committee (North West University ethics approval no. NWU-00255-17-S5). For this study, leopards are considered to be captive if they were either born in captive areas or raised from as early as 1 month of age.

Wild leopards sampling

The same sedation and sampling procedures followed during CapL data collection was applied for the blood collection of WL (Wild Leopards) at Mpumalanga and Northern Limpopo provinces of South Africa. These study subjects are considered to be WL if they live (born or caught at an adult stage in the wild) or when held at rehabilitation centres for less than six months to be relocated, but mainly if they were not introduced by humans at any area.

Blood collection procedure

Blood was collected from the jugular or cephalic vein using venipuncture with the use of sterile Vacutainer system in BD Vacutainer (Franklin Lakes, USA.) CAT (Clot Activator Tubes) and Vacutainer EDTA tubes for molecular analysis. Blood droplets were placed onto clean, pre-labelled microscope slides to make thin blood smears. Blood smears were air-dried and subsequently fixed with absolute methanol for one minute. Air-dried smears were then stained with Giemsa solution (prepared with distilled water (ratio of 9:1) in a 50ml staining container) for 20 minutes and then left to dry again.

Lab procedure

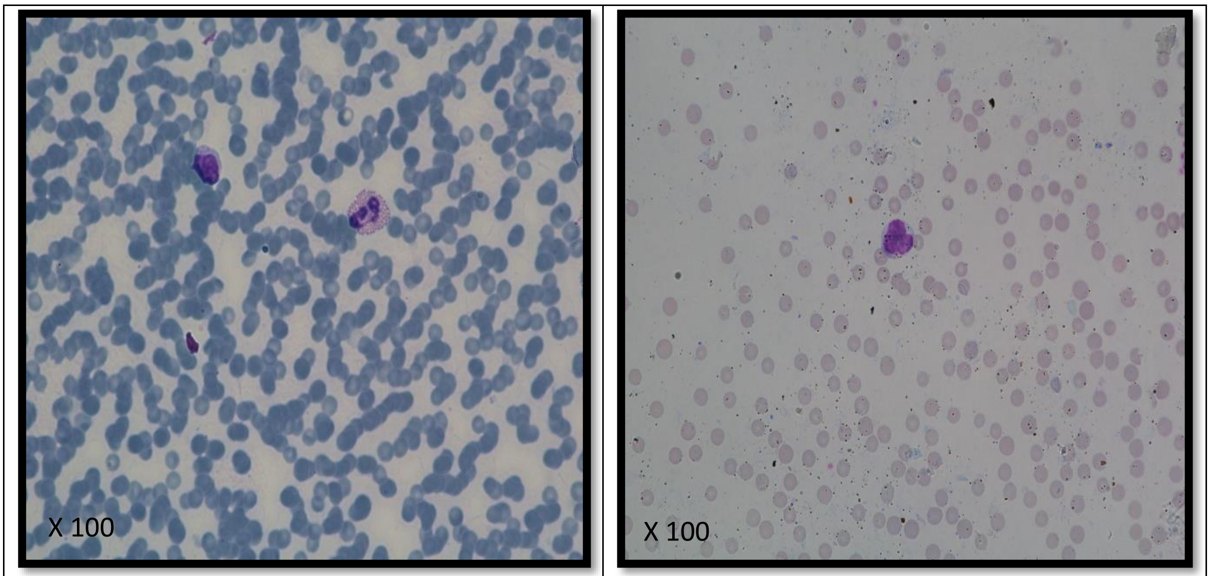
Micrographs were taken under the 100x oil immersion objective of a Nikon Eclipse E800 compound microscope (Nikon, Amsterdam, The Netherlands)

Blood count procedure

White Blood Cells (WBC) and Red Blood Cells (RBC) counts were done using ImageJ version 1.47 software program (Wayne Rasband National Industries of Health, USA.) (http://image.nih.gov/ij). For captive leopards, a maximum of 15 fields (micrographs) were counted, while 21 fields were not exceeded when doing blood counts for wild leopards. An hp laptop was used during the blood counts.

Identification of blood cells

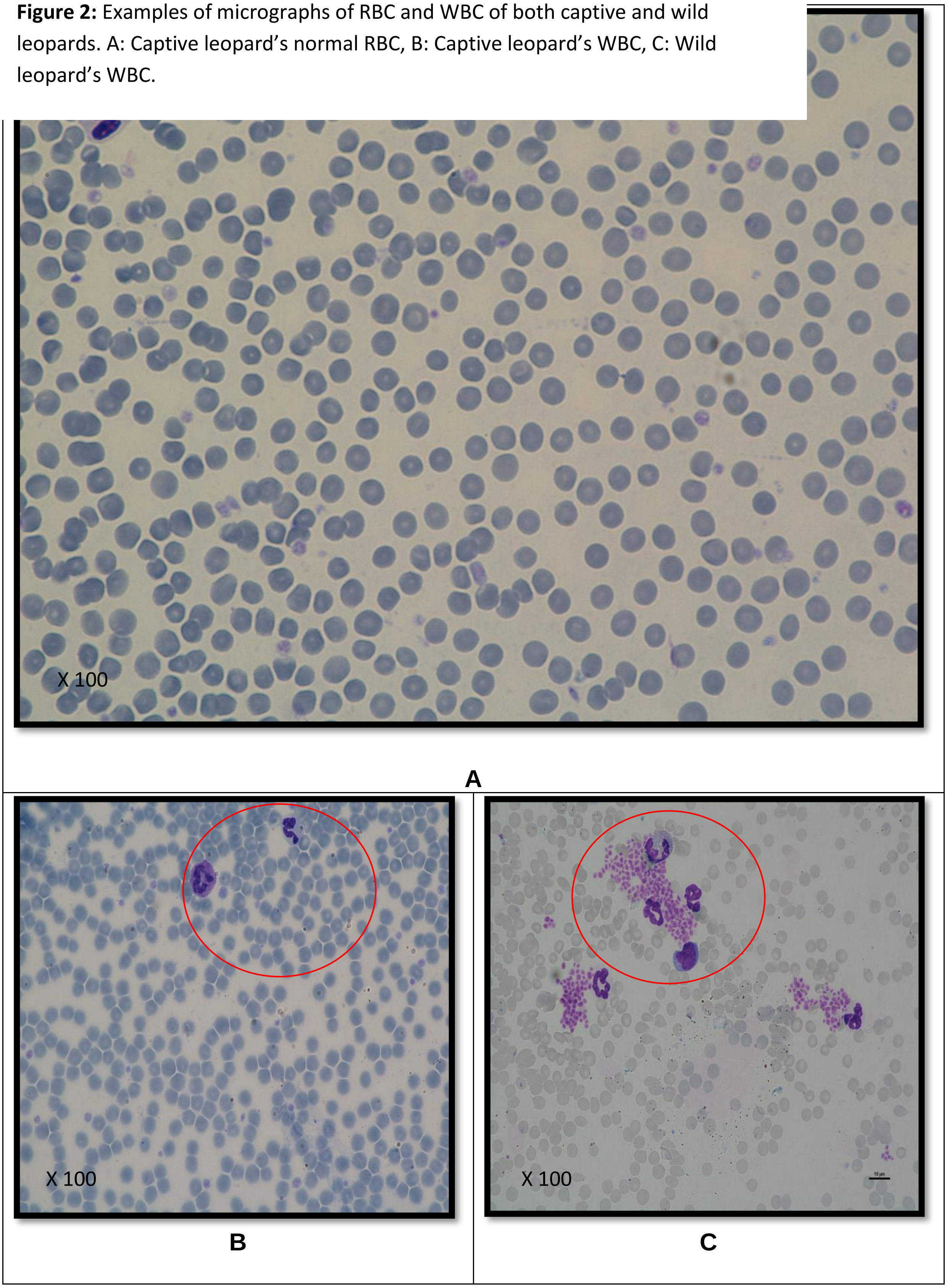
The Atlas of Canine and Feline peripheral blood smears, edition 1 (Valenciano et al., 2014) was used for identification of different blood cells. The following micrographs were identified using the above mention source:



A	**B**

Figure 1: Examples of micrographs of RBC of both captive and wild leopards A: Captive leopard RBC showing Rouleaux, B: Wild leopard's normal RBC.

Figure 2: Examples of micrographs of RBC and WBC of both captive and wild leopards. A: Captive leopard's normal RBC, B: Captive leopard's WBC, C: Wild leopard's WBC.



Statistical analysis

Ratios were calculated using an online ratio calculator called Calculator soup (Calculator Soup 2020). The calculator Soup calculates ratios that were already in lowest terms, for instance the total ratio of wild female leopards (See table 6). To get to the above mentioned ratio, both the terms were multiplied by 2 (Calculator Soup 2020). Furthermore, it is able to determine the ratio of values that are not in the lowest terms, by dividing the ratio with the greatest common factor (GCF). For instance, the total ratio of female captive leopards (see table 6) was determined using such a method.

For the purpose of determining whether all the total ratios are equivalent to each other, the calculator will find the values of A/B and C/D and compare the results to evaluate whether the statement is true or false (Calculator Soup 2020).

Results

Table 4: Ratios of captive VS. Wild leopards

Captive leopards			Wild leopards		
Study subjects	Actual WBC:RBC ratio	Standardized WBC:RBC ratio	Study subjects	Actual WBC:RBC ratio	Standardized WBC:RBC ratio
Cap L 1	76:13894	5.47:1000	W L 1	41:3764	1.09:1000
Cap L 2	40:11478	3.48:1000	W L 2	54:4514	1.19:1000
Cap L 3	38:16670	2.28:1000	W L 3	46:12350	3.72:1000
Cap L 4	1:347	2.89:1000	W L 4	74:18898	3.91:1000
Cap L 5	58:13226	4.39:1000	W L 5	122:13390	9.11:1000
Cap L 6	20:1711	1.17:1000	Total ratio	542:71736	7.55:1000
Cap L 7	46:12300	3.73:1000			
Cap L 8	26:11888	2.19:1000			
Cap L 9	5:3504	1.43:1000			
Total ratio	470:123238	3.81:1000			

Captive leopards have a lower WBC to RBC ratio (3.81:1000) than that of wild leopards (7.55:1000).

le leopards

Study subjects	Actual ratio WBC:RBC	Standardized ratio WBC:RBC
Females	31:8470	3.7:1000
Males	222:55478	4:1000

adults leopards

Study subjects	Actual ratio WBC:RBC	Standardized ratio WBC:RBC
Adults	282:75278	3.74:1000
Sub-adults	47:11990	4:1000

For captive leopards, males have a higher WBC: RBC ratio (4:1000) than that of females (3.7:1000). However, there is only a 0.3 difference between the two ratios. When comparing ratios of adults against those of sub-adults in captive leopards, one finds a much similar ratios to that of female against male leopards. Adults have a 3.74:1000; whereas sub-adults have 4:1000 WBC to RBC ratio. With only 0.26 as the difference between the two (See table 6)

Table 7: Ratios of wild females and male leopards

Study subjects	Actual ratio WBC:RBC	Standardized ratio WBC:RBC
Females	364:39448	9.23:1000
Males	49:48072	1.02:1000

Table 8: Ratios of wild adults and sub-adults leopards

Study subjects	Actual ratio WBC:RBC	Standardized ratio WBC:RBC
Adults	374:45996	8.13:1000
Sub-adults	14:2145	7:1000

For wild leopards, females have a higher WBC: RBC standardized ratio as 9.23:1000 is higher than that of 1.02: 1000 (See table 7). When comparing wild adults to sub-adults, we see a small difference between the two ratios (1.13) as adults have 8.13: 1000 as their ratio and sub-adults have 7:1000 WBC to RBC ratio. The data simply indicates adults as having a higher WBC to RBC ratio than that of captive sub-adults.

For comparisons between captive and wild female leopards, one can notice that wild female leopards have a higher WBC to RBC ratio than that of captive female leopards. With 9.23:1000 as the higher ratio and 3.7:1000 represents captive leopards. This is where a much bigger difference is observed as 5.53 is the differentiating value. The counterparts (males), do not show that much of a difference as captive male leopards have a higher WBC to RBC ratio (4:1000) than that of wild male leopards (1.02:1000). In both comparisons between captive adults against wild adults and captive sub-adults and wild sub-adults, wild leopards have a higher WBC: RBC ratio to that of captive leopards (see table 6 and 8).

Discussions

The current study's results indicate either a small or huge differences between the WBC to RBC ratio of leopards. The difference is not only seen when comparing captive against wild leopards, but also when comparing captive leopards to each other, for instance, female captive leopards have a different WBC to RBC ratio than that of captive males. Such results (difference between ratios) indicating that females are healthier than males could be because of a wider spectrum of prey species/diet than males (Voigt et al. 2018, Forschungsverbund Berlin 2018). Although these females are captive, for instance inhabiting a wildlife sanctuary, when fed, they will tend not to be choosy and eat what is served.

Wild females on the other hand have a higher WBC to RBC ratio than that of males, which indicates that they are not as healthy as males. Based on Voigt et al. (2018) and Forschungsverbund Berlin (2018), wild females will be more in high risk of getting diseases and infections as they fed on a large variety of prey species. A prey species might have diseases, or it might have venom/poison or the female leopard might have to endure a long agonising hunt full of traps and enemies, which will in turn increase the WBC, which are for immunity purposes.

Generally, wild leopards have a higher WBC to RBC ratio than that of captive leopards. This simply indicates and proves that captive leopards are generally healthier than wild leopards. This could be due to factors mentioned above based on females, as there are more studied wild female leopards than wild male leopards. Either way, being in the wilderness might be natural for wild animals; however, the increase in mortality rates due to poor health indicates that it is not an option any longer.

In the wild, adult leopards are not healthy, when comparing them to sub-adults; however the 1.13 difference could be because sub-adults also get involved in hunting (Sunquist &Sunquist 2017) making them more susceptible to diseases and infections.

This study has generally indicated that captive leopards are healthier than wild leopards, although there are instances where by a certain sex of wild leopards is indicated to be healthier than captive leopards. These results also prove that my hypothesis correct and also send a message to the South African government to develop more protected areas for leopards. More research, however, should be conducted in order to widen research on big cats' health based on hematological values.

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