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Research Article

Turk. J. Vet. Anim. Sci.
2012; 36(4): 346-351
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doi:10.3906/vet-1102-757

Comparative hematological variables of Bengal tigers (*Panthera tigris tigris*) kept in Lahore Zoo and Lahore Wildlife Park, Pakistan

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Received: 03.02.2011 ● Accepted: 23.09.2011

Abstract: In Pakistan, a scant number of Bengal tigers (*Panthera tigris tigris*) are being kept at various zoos or in certain wildlife sanctuaries, and there is a dearth of literature regarding the effect of captivity on their physiological parameters. The present study was hence conducted to determine and compare the hematological alterations in tigers of Bengal origin (*Panthera tigris tigris*) kept in captivity at Lahore Zoo (LZ) (n = 4) and in the seminatural environment of Lahore Wildlife Park (LWP) (n = 6), Pakistan. The results reflect a significantly higher ($P < 0.05$) total leucocyte count and platelet count and a lower packed cell volume in animals kept at LWP as compared to those of animals kept at LZ. No other significant differences in hematology were noted for the 2 captive forms. Despite the limitation of sample size in the present study, it was concluded that captivity has a significant effect on various hematological parameters in Bengal tigers. These baseline data will lay a foundation in the therapeutic evaluation and physical monitoring of tigers kept in various forms of captivity not only in Pakistan, but also in other countries such as India, Nepal, Bhutan, and Bangladesh, as well.

Key words: Hematology, captivity, *Panthera tigris tigris*

Introduction

In response to the loss of many species worldwide, the approach of conservation has been directed toward the introduction of various captivity modules such as zoos, sanctuaries, and wildlife parks (1). Apart from serving the main aim of conservation, these modules also serve as a seat of education, research, and recreation (2). However, the captive, human-made environments are not suitable for the animals to carry out their natural instinctive behaviors, and they hence result in physiochemical alterations aimed toward the adaptation of homeostatic challenges (3).

The tiger is the largest of the cats (4,5) and is one of the most magnificent animals. There are a total of 9 subspecies of tigers (Table 1), out of which 3 have already become extinct. The Bengal tiger (*Panthera tigris tigris*) has recently been categorized as globally endangered (6). In Pakistan, a scant number of Bengal tigers are being kept at various zoos or in certain wildlife sanctuaries, and the effect of captivity on their physiological variables has not yet been published. Hence, the objectives of this study were to determine and compare the hematological profiles in tigers of Bengal origin (*Panthera tigris tigris*) kept in captivity at Lahore Zoo (LZ) and in

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Table 1. The subspecies of tiger and their geographic distribution (6).

Species	Subspecies		Geographical distribution
	Common name	Scientific name	
Panthera tigris (P.t)	Bengal tiger	*P.t. tigris*	Indian subcontinent
	Caspian tiger	*P.t. virgata*‡	Formerly in Turkey through central and western Asia
	Amur tiger	*P.t. altaica*	Amur River region of Russia, China, and Korea
	Javan tiger	*P.t. sondaica*‡	Formerly in Java
	South China tiger	*P.t. amoyensis*	South-central China
	Bali tiger	*P.t. balica*‡	Formerly in Bali, Indonesia
	Sumatran tiger	*P.t. sumatrae*	Sumatra, Indonesia
	Indo-Chinese tiger	*P.t. corbetti*	Continental southeastern Asia
	Peninsular tiger	*P.t. jacksoni*	Peninsular Malaysia

‡ Extinct subspecies.

the seminatural environment of Lahore Wildlife Park (LWP), Pakistan. The results would be useful for the evaluation of physiological and pathological alterations in wild and captive tiger individuals and populations in Pakistan and other countries harboring Bengal tigers.

Materials and methods

Study area and experimental animals

The present study was conducted on the tigers of Bengal origin (*Panthera tigris tigris*) kept at LZ, Mall Road, Lahore, and at LWP, located on Raiwind Road 32 km from the main city of Lahore, in 2007 and 2008. The tigers of LZ (n = 4) live in solitary and consist of 2 males and 2 females, whereas the tigers of LWP (n = 6) live in social groups and consist of 5 males and 1 female. All of the animals ranged from 1 to 4 years in age and from 140 to 170 kg in weight.

Housing and feeding

The Bengal tigers housed at LZ in captivity were kept in indoor enclosures (approx. 7.6 × 3 × 4.6 m) with wired fence on the front and back for the provision of natural light. The floor was tiled and there were solid opaque walls on the sides, which prevented physical and visual contact with animals housed in adjacent cages. All of the cages were provided with fans, air coolers, and a water pool with ad libitum water. On the other hand, the tigers dwelling in the seminatural environment of LWP were kept in the outdoor enclosures with ample space (6.07 ha) and were provided with dense vegetation consisting of trees, bushes, and grass. Man-made hills and hideouts were also provided as natural housing for the tigers.

The tigers were fed a 24-h standard diet consisting of 1 L of milk and 7-8 kg of meat. None of the tigers were ill or involved in any other study/trial that might have influenced this study.

Standard capture and sampling protocols

Standard capture protocol was used and observed at both sites of study. This involved herding the animals to a corner of their enclosure with the help of hand-operated doors and directional iron rods, and finally capturing the animals in squeeze cages (2.4 × 2.1 × 2.4 m). After squeezing in, the animals were allowed to settle down in order to normalize their body temperatures and heartbeats. From the common tail vein (dorsal coccygeal vein), 5 mL of blood was collected aseptically using disposable syringes and 23-gauge needles, and the blood was then transferred into screw-capped tubes containing 0.5 mL of 1% EDTA solution as an anticoagulant for hematological analysis. In order to minimize the stress in the animals, the collection procedure was standardized

by using the same personnel, same technique of restraint, and same time of the day (between 0900 and 1200 hours). Blood samples were transported in an ice box to the Department of Zoology of the Lahore College for Women University in Lahore for analysis. Each animal was bled thrice and a total of 30 samples (12 from LZ captives and 18 from LWP animals) were collected.

Hematological analyses

The hematological values of total leukocyte count (TLC), total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and total platelet count (TPC) were determined using a Sysmex hematology analyzer (Model-KK-2; New Jersey, USA).

Statistical analysis

Data were expressed as mean, standard deviation, and standard error of mean (±SEM). Following homogeneity of variance, comparisons among and between animals kept in captivity at LZ and LWP were made using ANOVA and t-tests with Microsoft Office Excel 2000.

Results

Mean ± SEM hematologic variables for tigers confined in captivity at LZ are presented in Table 2, and for those confined at LWP they are given in Table 3. The hematologic analysis revealed that the tigers kept at LWP had significantly ($P < 0.05$) higher mean concentrations of TLC (16.62 ± 0.67 versus 9.15 ± 2.37 × 10^3/μL) and TPC (263.9 ± 11.3 versus 165.6 ± 30.48 × 10^3/μL) and a significantly lower PCV (52.94 ± 2.93% versus 61.80 ± 1.83%) than tigers kept at LZ. All other hematologic parameters were found to be nonsignificant between and within the tigers of both captive forms (Table 4).

Discussion

The hematologic values presented in the present study include most of the tests of interest in a routine clinical pathology laboratory. Though the population sample is small, for wildlife species it chalks out

Table 2. Mean (± SE) hematological values in tigers confined to captivity at Lahore Zoo (LZ).

Parameters	Animal I.D.				Mean ± SE (range)
	801	802	803	804	
Total erythrocyte count (10^6/μL)	10.32 ± 2.81	10.40 ± 2.30	9.60 ± 2.01	11.00 ± 2.48	10.33 ± 2.40 (9.60-11.0)
Hemoglobin (g/dL)	14.69 ± 0.30	15.76 ± 0.82	14.83 ± 0.60	13.56 ± 0.80	14.71 ± 0.63 (13.56-15.76)
Packed cell volume (%)	61.78 ± 13.02	65.46 ± 11.5	59.60 ± 11.40	60.36 ± 11.40	61.80 ± 11.83 (59.6-65.46)
Mean corpuscular volume (fL)	63.68 ± 2.42	64.03 ± 3.90	62.46 ± 1.07	64.63 ± 6.19	63.70 ± 0.64 (62.46-64.63)
Mean corpuscular hemoglobin (pg)	18.92 ± 0.37	18.93 ± 0.38	18.83 ± 0.29	19.00 ± 0.34	18.92 ± 0.04 (18.83-19.0)
Mean corpuscular hemoglobin concentration (g/dL)	28.78 ± 1.75	29.90 ± 2.40	30.16 ± 0.80	26.36 ± 0.44	28.80 ± 1.22 (26.36-30.16)
Total leukocyte count (10^3/μL)	9.15 ± 2.25	9.53 ± 2.86	13.06 ± 2.61	4.86 ± 1.21	9.15 ± 2.37 (4.86-13.06)
Total platelet count (10^3/μL)	165.5 ± 68.47	120.30 ± 71.8	223.60 ± 73.05	153.00 ± 45.54	165.6 ± 30.48 (120.3-223.6)

Table 3. Mean (± SE) hematological values in tigers confined to captivity at Lahore Wildlife Park (LWP).

Parameters	Animal I.D.						Mean ± SE (range)
	8A	8B	8C	8D	8E	8F	
Total erythrocyte count (10^6/μL)	8.43 ± 0.60	7.64 ± 1.76	10.65 ± 2.18	12.20 ± 1.90	11.00 ± 1.58	9.00 ± 0.31	9.92 ± 0.75 (7.64-12)
Hemoglobin (g/dL)	16.06 ± 1.09	14.50 ± 3.10	14.63 ± 0.85	15.33 ± 0.66	14.73 ± 0.63	15.13 ± 0.52	15.06 ± 0.23 (14.5-16.06)
Packed cell volume (%)	50.90 ± 3.81	47.00 ± 8.42	66.23 ± 13.15	46.46 ± 0.69	53.03 ± 1.07	54.03 ± 0.54	52.94 ± 2.93 (46.46-66.23)
Mean corpuscular volume (fL)	60.40 ± 1.85	60.03 ± 3.46	62.33 ± 1.57	51.93 ± 1.03	61.90 ± 0.77	62.83 ± 62.83	60.40 ± 1.73 (51.93-62.83)
Mean corpuscular hemoglobin (pg)	19.06 ± 0.14	19.16 ± 0.46	18.00 ± 0.26	14.83 ± 0.90	17.13 ± 0.52	19.00 ± 0.05	17.86 ± 0.68 (14.83-19.16)
Mean corpuscular hemoglobin concentration (g/dL)	31.66 ± 1.19	30.56 ± 1.36	28.86 ± 0.84	31.46 ± 1.34	30.03 ± 0.52	28.46 ± 0.52	30.17 ± 0.53 (28.46-31.66)
Total leukocyte count (10^3/μL)	15.93 ± 2.59	18.43 ± 5.91	14.06 ± 4.58	16.00 ± 1.08	18.40 ± 0.78	16.00 ± 1.02	16.62 ± 0.67 (14.06-18.43)
Total platelet count (10^3/μL)	282 ± 9.29	263.3 ± 36.7	247.60 ± 59.52	237.00 ± 32.90	246.00 ± 14.22	309.6 ± 1.45	263.9 ± 11.3 (234-309)

baseline data for further extensive studies. To date, there is no published hematologic data for Bengal tigers kept in captivity in Pakistan.

The mean values of TEC recorded in this study were 10.33 ± 0.40 and 9.92 ± 0.75 × 10^6/μL for LZ and LWP captive tigers, respectively, and were statistically nonsignificant. These values were slightly higher than the mean TEC values of 7.17 ± 0.33 and 7.05 ± 0.12 × 10^6/μL reported by Singh et al. (7) for male and female Bengal tigers in India, respectively. Similarly, lower values of 6.5 and 6.1 × 10^6/μL have been reported by Jain (8) and Ulysses et al. (9), respectively. Higher TEC values in this study may be attributed to variable age, physiologic state, stress, and climate. Furthermore, some hematology analyzers are unable to accurately count feline red blood cells (RBCs), which can be very small (10).

Blood Hb concentrations for LZ and LWP captive tigers recorded in this study were 14.71 ± 0.63 and 15.06 ± 0.23 g/dL, respectively, and were in line with the mean value of 15.75 ± 1.40 g/dL reported by Singh et al. (7) for Bengal tigers in India. However, a slightly lower mean value of 12.1 g/dL was reported by Jain (8). Higher values in Pakistan and India may be because of the variation in climatic condition. Animals in hot tropical countries have been shown to have a higher TEC and Hb level, indicating a physiological adaption and not a pathological state (11).

The mean values of PCV recorded in this study for tigers kept at LZ and LWP were 61.80 ± 1.83% and 52.94 ± 2.93%, respectively, and were significantly higher for LZ captive tigers than their counterparts. Better nutritional/feeding system efficiency and general health status of the animals may be correlated to higher values in LZ-kept tigers, as this parameter is a suitable indicator of nutritional state (12). These values, however, do not correlate with most of the previous work done. A significantly lower value of 45.67 ± 2.42% was reported by Singh et al. (7). Similarly, a mean PCV value of 37% was reported by Jain (8). The PCV percentage was analyzed with a hematology analyzer, which can give improper readings if not calibrated from time to time for the species in question (8).

Table 4. Comparative mean (± SEM) hematological values in tigers confined to captivity at Lahore Zoo (LZ) and Lahore Wildlife Park (LWP).

Parameters	Tigers at LZ (n = 4)	Tigers at LWP (n = 6)
	Mean ± SE (range)	Mean ± SE (range)
Total erythrocyte count (10^6/μL)	10.33 ± 0.40 (9.60-11.0)	9.92 ± 0.75 (7.64-12)
Hemoglobin (g/dL)	14.71 ± 0.63 (13.56-15.76)	15.06 ± 0.23 (14.5-16.06)
Packed cell volume (%)	61.80 ± 1.83* (59.6-65.46)	52.94 ± 2.93 (46.46-66.23)
Mean corpuscular volume (fL)	63.70 ± 0.64 (62.46-64.63)	60.40 ± 1.73 (51.93-62.83)
Mean corpuscular hemoglobin (pg)	18.92 ± 0.04 (18.83-19.0)	17.86 ± 0.68 (14.83-19.16)
Mean corpuscular hemoglobin concentration (g/dL)	28.80 ± 1.22 (26.36-30.16)	30.17 ± 0.53 (28.46-31.66)
Total leukocyte count (10^3/μL)	9.15 ± 2.37* (4.86 -13.06)	16.62 ± 0.67 (14.06-18.43)
Total platelet count (10^3/μL)	165.6 ± 30.48* (120.3-223.6)	263.9 ± 11.3 (234-309)

Values with an asterisk in a row differ significantly at $P \leq 0.05$ from those of the LWP animals.

All of the erythrocyte indices (MCV, MCH, and MCHC) were nonsignificant for LZ- and LWP-kept tigers. The mean MCV values for tigers kept at LZ and LWP recorded in the study were 63.70 ± 0.64 and 60.40 ± 1.73 fL, which were in line with the mean value of 63.70 ± 1.45 fL reported by Singh et al. (7) for Bengal tigers in India. A relatively lower mean MCV value of 56.1 was reported by Jain (8). The mean MCH values for LZ- and LWP-kept tigers of the present study (18.92 ± 0.04 and 17.86 ± 0.68 pg, respectively) were lower than the value of 22.07 ± 0.90 pg reported by Singh et al. (7). However, our values were in line with that of 18.4 pg reported by Jain (8). The mean MCHC vales of the present study were slightly lower than that reported by Singh et al. (7) of 34.70 ± 1.60 g/dL and that reported by Jain (8) of 32.70 g/dL. The variation in various erythrocyte indices may be attributed to variable RBC size and its oxygen carrying capacity in connection with age and physiologic state. Difference due to variance in the technique used can also not be ruled out.

The high TLC concentration in the tigers kept in the seminatural environment of LWP as compared to those kept in captivity at LZ in the present study was in line with the findings of Weaver and Johnson (13), who reported a high TLC of 14.67 ± 4.07 × 10^3/μL in captive wild lynx and correlated it to the 'capture stress'. The tigers kept at LZ have presumably accustomed themselves to captivity; hence, their TLC concentrations were not as elevated as expected. The mean TLC values of the present study are in accordance with the reference TLC value of 16,600 reported by Jain (8) for 3-year-old male Bengal tigers and that of 10.5 ± 3.1 × 10^3/μL reported by Ulysses et al. (9) for Bengal tigers immobilized by ketamine and xylazine. The TLC, however, usually demonstrates the greatest variations in comparisons of different studies within the same species (14).

The important role that platelets play in the hemostatic mechanism of the body has been well documented (7,15). Knowledge of their structure and function is therefore important as it may be the first indication of an underlying clinical condition or pathology that may lead to defective hemostasis. The mean TPC values for LZ and LWP tigers were 165.630.48 and 263.9 ± 11.3 × 10^3/μL, respectively, and were statistically higher in LWP-kept tigers. Stress or excitement at the time of sampling, a

phenomenon known as the 'epinephrine effect', may be attributed to this high TPC in LWP-kept tigers (16). The TPC values of the present study could not be compared with any earlier work because of the lack of published data regarding platelet count in tigers. However, these values were lower than those reported for certain other felids. Dunbar et al. (17) and Foster and Cunningham (18) reported TPC values of 402.6 ± 31.5 and 605.8 ± 72.8 × 10^3/μL, respectively, for adult Florida panthers (*Felis concolor*). Similarly, a mean TPC value of 275.5 ± 43.6 × 10^3/μL for lions (*Panthera leo*) was reported by Du Plessis (19). The variation can be attributed to the inability of hematology analyzers to accurately count feline platelets, which can be very large (10).

The present study clearly indicates that environment, whether captive or seminatural, has a significant effect on many hematological variables. The total lack of published literature regarding the effect of captivity on physiological variables for tigers in Pakistan makes this preliminary study one of a kind and supports the need for further studies with larger populations and samples. The results would be useful for the evaluation of physiological and pathological alterations in wild and captive tiger individuals and populations in Pakistan and many other countries such as India, Nepal, Bhutan, and Bangladesh. Furthermore, the results of the study can also be used as baseline reference hematological variables in species under study.

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