

EVALUATION OF GENERAL HEALTH STATUS OF SOME AGED TIGRESSES AT A LOCAL CIRCUS, CAIRO, EGYPT

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ABSTRACT

The present study was undertaken to evaluate the general health status of three aged female tigers around 20 years old at a local circus in Egypt for the first time. Tigers (*Panthera tigris*) are one of the largest Felidae species, which are classified as Endangered species. Blood and fecal samples were collected for hematological and biochemical analysis, parasitological and bacteriological examination. The hematological results revealed macrocytic normochromic anemia and non-significant changes in leucocyte and differential leucocytic count. The biochemical analysis showed a normal level of liver enzymes. Blood urea nitrogen (BUN), creatinine (CREA), chloride (Cl⁻), and cholesterol were higher than normal. On the other hand, normal Glucose (GLU), Calcium (Ca), Phosphate (PHOS), Sodium (Na⁺), and Potassium (K⁺) were observed. In addition, low levels of total carbon dioxide (tCo₂) and platelets. The parasitological examination of fecal samples was negative, and tigresses were free from any external or internal parasitic infestation. Advanced studies are required to cover the complete picture of tiger status in captivity.

Keywords: Circus, parasites, felids, captive, hematology.

INTRODUCTION

Wild animals are usually kept in captivity in zoos or circuses and in close contact with humans, especially caretakers, hence serving as indicators of human risk of exposure to zoonotic agents and representing a source of infection for humans and other animals (Iatta *et al.*, 2020). Tigers (*Panthera tigris*) are one of the largest Felidae species. They are classified by International Union for

the Conservation of Nature as endangered with a decreasing population trend and listed on its red list of threatened species (Goodrich *et al.*, 2015). Tigers are further listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). These species are especially susceptible to metabolic and behavioral changes related to a change in their natural conditions, habitat, and feeding when the animal enters into captivity (Larsson *et al.*, 2017).

Less than 3500 tigers now live in the wild. This risky situation gives the health of each individual tiger the maximum importance, so

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health monitoring is vital (Proverbio *et al.*, 2021). The health of different animals can be assessed through hematological and biochemical parameters. Nevertheless, reports on tigers' hematological and biochemical parameters are very limited (Liu *et al.*, 2021). Because obtaining biological samples from free-living tigers is very difficult, therefore, the data obtained from captive tigers are very useful (Proverbio *et al.*, 2021).

To the best of the author's knowledge, this study was carried out for the first time on captive wild tigers kept at circus in Cairo, Egypt. Our aims are to safeguard the health status of three aged female South China tigers present in a local circus, through the evaluation of some hematological, biochemical parameters and parasitological examination.

MATERIALS AND METHODS

Animals: Three female South China tigers (*Panthera tigris var amoyensis*), around 18-20 years old, weighed about 90-100 kg, were living individually in enclosures. They feed every other day. The protocol of this study was approved by the ethical committee of the Faculty of Veterinary Medicine, Suez Canal University (No. 2021031).

Sampling and analysis:

1-Blood samples: 2 ml of whole blood was collected from the saphenous vein during the routine clinical examination at the squeeze cage in vacuum blood tubes containing EDTA for hematological analysis. Another 5 ml of blood was collected in plain tubes, incubated at 25°C for 30 min to clot, and then centrifuged at 3000 rpm for 15 min until serum was separated. The serum was kept at -20 °C before being processed for biochemical analysis.

2-Hematological analysis: The hematological parameters evaluated were: White blood cells (WBC), Lymphocytes (LYM), Monocytes (MON), Neutrophils

(NEU), Eosinophils (EOS), Basophils (BAS), Red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red blood cell volume distribution width (RDW), Platelet (PLT) and Mean platelet volume (MPV). The hematological analysis was achieved according to VetScan®HM5 and VETSCAN HM5 Operator Manual, LBL-03063, Zoetis, Inc.

3-Biochemical analysis: the measured biochemical parameters were: Alkaline phosphatase (ALP), Alanine transaminase (ALT), Total bilirubin (TBIL), Albumin (ALB), Blood urea nitrogen (BUN), Cholesterol (CHOL), Glucose (GLU), Creatinine (CREA), Calcium (Ca), Phosphate (PHOS), Sodium (Na⁺), Potassium (K⁺), Chloride (Cl⁻) and total Carbon dioxide (tCo₂). The biochemical analysis was done according to VetScan®VS2, Zoetis, Inc.

4-Fecal samples: Fecal samples were collected directly from the enclosure immediately after defecation, stored in a sterile universal collector, immediately identified, and stored under refrigeration at 3-5°C. A fresh fecal analysis was performed within 24 hours after harvest, in the laboratory of wildlife and zoo, faculty of veterinary medicine, Suez Canal University.

5-Parasitological analysis: Following the guidelines of (Carvalho *et al.*, 2018 and De Carli 2001), samples of fresh feces were taken from the collected specimens. First, they were diluted with 1mL buffered saline solution between the slide and cover slip and examined by the direct method of observation under the microscope. The Willis method consists of the process of fluctuating a portion of feces diluted in hyper-saturated saline solution while resting on the slides for 5 minutes, conditioned in a Petri dish. Three slides were prepared from each technique, stained with Lugol's, and studied under an optical microscope.

6- Data Analysis: Microsoft Excel® was used to enter and capture data. Various tables were extracted from this data. Data was then exported to SPSS for further analysis. It was analyzed using one-way analysis of variance (ANOVA). SPSS 15.0 was employed for statistical analysis.

RESULTS

During the routine clinical examination of aged female tigers at the national circus, we observed they were apparently healthy, but showed some emaciation. Regarding the parasitic examination, all tigresses were free from any external or internal parasitic infection.

According to Vetscan®HM5 Reference Ranges; the hematological finding revealed that WBC, Lymphocytes, Monocytes,

Neutrophils, Eosinophils, Basophils, and RBC were within the normal range. While HGB, HCT, MCV, MCH, and MPV were higher than normal. On the other hand, platelets (PLT) were lower than the normal level (Table 1).

According to Vetscan®VS2 Reference Ranges; the liver profile shown in (Table 2), ALP, ALT, Total protein, Total Bilirubin (TBIL), and Albumin (ALB) were normally presented as compared to the reference range. Cholesterol (CHOL) was higher than normal.

The kidney profile includes normal GLU, CA, ALB, PHOS, NA⁺, and K⁺ in comparison to the reference range. On the other hand, BUN, CRE, and CL⁻ were higher than normal, in addition to the lower level of tCO₂ (Table, 2).

Table 1: Complete blood count in female tigers.

Parameters	Units	Mean ± SE	Min- Max	Ref. Range
WBC	10 ⁹ /l	14.45±1.15	12.45-16.45	5.5-19.5
Lymphocytes	10 ⁹ /l	2.76±0.46	1.96-3.55	1.5-7.0
Monocytes	10 ⁹ /l	0.65±0.064	0.54-0.76	0.0-1.5
Neutrophils	10 ⁹ /l	10.85±0.78	9.5-12.2	2.5-14.0
Eosinophils	10 ⁹ /l	0.18±0.017	0.15-0.22	0.0-1.0
Basophils	10 ⁹ /l	0.01±0.003	0.005-0.015	0.0-0.2
RBC	10 ¹² /l	7.28±1.19	5.22-9.34	5.0-10.0
HGB	g/dl	16.7±0.88	15.19-18.22	8.0-15.0
MCV	fl	52.25±4.63	44.00-60.00	39.0-55.0
MCH	pg	23.00± 0.78	20-26	12.5-17.5
MCHC	g/dl	31.67± 0.78	30.30-33	30.0-36.0
PCT	%	0.40±0.12	0.20-0.60	0.22–0.24%
Platelet	10 ⁹ /l	232.00±10.7	222-242	300.0- 800.0
RDWCV	%	16.50± 0.87	15.00-18.00	12.0-17.0%
RDWSD	fl	42.20±1.04	40.40-44.00	40.0 - 55.0
PDWCV	%	38.90±1.44	36.40-41.40	8.3% - 56.6%
PDWSD	fl	16.00±1.15	14.00-18.00	16-24

Table 2: Some biochemical parameters in female tigers.

Parameters	Mean \pm SE	Min- Max	Ref. Range
ALT (u/l)	68 \pm 4.62	60.00-76.00	20.0-100.0
Total protein (g/dl)	6.50 \pm 1.10	4.60-8.40	3.7–8.7 U/L
Albumin (g/dl)	4.16 \pm 0.15	3.900-4.400	2.2-4.4
BUN (mg/dl)	41.00 \pm 1.15	39.00-43.00	10.0-30.0
Creatinine (mg/dl)	5.80 \pm 0.81	4.400-7.200	0.3-2.1
Total Bilirubin (mg/dl)	0.30 \pm 0.115	0.10-0.50	0.1-0.6
ALP (u/l)	32.00 \pm 5.77	22.00-42.00	0.0-90.0
CHOL (mg/dl)	267.00 \pm 7.51	254.00-280.00	90.0-205.0
CL- (mmol/l)	125.00 \pm 2.89	120.00-130.00	99.0-122.0
GLU (mg/dl)	89.00 \pm 5.20	80.00-98.00	70.0-150.0
CA (mg/dl)	10.43 \pm 1.47	7.90-13.00	8.0-11.8
PHOS (mg/dl)	5.20 \pm 1.04	3.40-7.00	3.4-8.5
NA+ (mmol/l)	153.00 \pm 5.20	144.00-162.00	142.0-164.0
K+ (mmol/l)	4.50 \pm 1.48	2.4-6.6	3.7-5.8
tCO ₂ (mmol/l)	12.00 \pm 1.73	9.00-15.00	15.0-24.0

DISCUSSION

Wild animals are good at hiding signs of illness, which may be because animals with obvious dysfunction will be recognized by predators and taken from the population (Wobeser 2013). Hematology provides an effective way of evaluating the physiological status of animals, and often gives the primary indicator of disease (Larsson *et al.*, 2015).

The hematological finding revealed that WBC, Lymphocytes, Monocytes, neutrophils, Eosinophils, Basophils, and RBC of tigresses were within the normal range. These results were parallel to those (Larsson *et al.*, 2015 and Liu *et al.*, 2021). While HGB, HCT, MCV, MCH, and MPV were higher than normal. These results coincide with (Currier and Russell 1982) who reported high levels of Pumas' hematocrit and hemoglobin since the animals were collected at various altitudes. Larsson *et al.* (2015) also added that higher values for such parameters were seen in animals at high elevations compared to those at sea level.

However, the high rise of packed cell volume might be associated with the habitat, management, physiological status, plan of nutrition, and environmental factors (Gad and Shyama 2009 and Sankar *et al.*, 2001). Apart from that, stress, excitement, and circadian and diurnal rhythms are prime factors, as they are responsible for variation in blood physiological parameters (Benjamin 1978). Shrivastav and Singh (2012) found PCV (36-45, 38 \pm 4.45%) in free-ranging tigers. The effects of PCV fluctuations are primarily limited to increased erythrocyte osmotic fragility in immune-mediated hemolytic anemia. Taketa *et al.* (1967) have assessed that the oxygen affinity of hemoglobin is much lower in felines than other mammals, including humans. Additionally, higher mean corpuscular hemoglobin (MCH) is reported, along with the most elevated MCV findings. MCV and/or MCH elevations point to macrocytosis. The presence of macrocytosis may not always call additional testing or medical intervention. But medical professionals need to be aware of the significance of macrocytosis in the probable emergence of clinically severe anemia (macrocytic anemia).

RDW represents the different values of circulating RBCs. High RDW is linked to several illnesses, including cancer, malnutrition, liver disease, and anemia brought on by a lack of iron or vitamin B₁₂, as well as hemolysis brought on by the destruction of RBCs (Tseliou *et al.*, 2014). Sarma (1990) stated that further aids in morphology-based anemia evaluation include research into the general availability of RDW as a measure of anisocytosis. The MCV and MCH are increased, while the MCHC remains normal. RDW is frequently elevated, and anisocytosis is present. In the macrocytosis of liver disease, whereas in iron deficiency, anisocytosis may be the initial laboratory anomaly even before anemia is observed. Chandranaik *et al.* (2006) also reported mild anisocytosis in physically restrained tigers. However, the range and mean (with one standard deviation) of total erythrocyte count (TEC) was 4.66-9.15, 7.9±1.42 million/ μ l. Likewise, hemoglobin concentration (Hb) was obtained at 9.8-13.5, 12.8 ±1.65 mg/dl in males and 7.8-11.5, 10.8±1.05 mg/dl in females (Shrivastav *et al.*, 2011).

MPV is a sign of bone marrow platelet production. In myeloproliferative illnesses, or immune thrombocytopenic purpura, the MPV rises, while in aplastic weakness, MPV diminishes due to decreased platelet formation. (Iida *et al.*, 2018). PLTs essentially serve a hemostatic function in the body. In this consideration, the PLT numbers were below the reference range. This may be due to the accumulation of PLTs in vitro and the comparable size of RBCs and PLTs, which led to a small number of PLTs being rejected by the programmed hematology analyzer (Norman *et al.*, 2001). Decreased PLT levels may be frequent observation in conditions such disseminated intravascular coagulation, severe leukemia, and idiopathic thrombocytopenic purpura. The greatest work of MPV is to recognize the source of thrombocytopenia (May *et al.*, 2019).

Increased BUN is frequently linked to hypertension, shock, congestive heart failure,

and renal disease, and might be due to high meat intake (Shrivastav and Singh 2012). Additionally, extra-renal variables such as dietary protein intake, dehydration, decreased cardiac output, and other diseases might have an impact on BUN (e.g., gastrointestinal hemorrhage, and pyrexia) (Mota *et al.*, 2021). Dehydration generally causes urea levels to rise more than creatinine levels, and this is a possible explanation for the higher urea concentration values found in our tigresses. Previous studies on gray wolves (Constable *et al.*, 1998) and European wild cats (Marco *et al.*, 2000) attributed the high urea values to the level of protein intake (Liu *et al.*, 2021 and Proverbio *et al.*, 2021). Moreover, adult tigers consumed more meat; this could be one of the reasons for the Creatinine level increasing with age (Liu *et al.*, 2021). Furthermore, blood creatinine concentration can be impacted by some extra-renal variables such as endogenous muscle synthesis, dietary protein intake, age, and sex (Mota *et al.*, 2021).

The high CL- value recorded in the kidney, could be due to the effect of captivity as mentioned by Farooq *et al.* (2012) while working on comparable serum chemistry values of Bengal tigers kept in different forms of captivity. Chronic renal failure is common in geriatric felids (Lamberski 2015).

Nevertheless, measuring AST and ALT levels is important and aids in identifying liver damage, skeletal muscle injuries caused by shoot immobilization, trap capture or restriction stretching (Seal *et al.*, 1987), cardiac muscle damage, and strenuous workout (Liu *et al.*, 2021). The liver profile in this study showed a normal level of ALP, ALT, Total Protein, Total Bilirubin (TBIL), and Albumin (ALB) as compared to the reference range. These results agree with the findings of (Jiang *et al.*, 2019) who stated that ALP levels were higher in younger tigers, as it is important for osteogenesis, mineralization and bone formation. The primary method for determining jaundice is the TBIL, and is a crucial indicator of liver health. The TBIL

concentrations in this investigation were within normal limits. Some cases, such as jaundice, hepatitis, and hepatonecrosis could result from higher TBIL levels (Liu *et al.*, 2021).

In the present work, CHOL level in tigresses was higher than normal. On the other hand, CHOL levels were significantly lower in captive Siberian tigers, due to minimum activity and limited confinement space (Liu *et al.*, 2021). According to Currier and Russell (1982), CHOL is regarded as an endpoint for animals held in captivity, who are supported by a distinct slim down compared to wild individuals of the same species, and who are housed in environments with lower mobility levels. The increase in lipoproteins is associated with increased cholesterol. This rise can happen as an essential condition, or optionally as in Cushing's disorder, high-fat diets, diabetes mellitus, hypothyroidism, starvation, and blockage of the bile conduits. Among the biochemical constituents, i.e. glucose, calcium, chloride, cholesterol, and blood urea, nitrogen levels were essentially higher in grown-ups as compared to sub-adult gaurs. (Zimmerman *et al.*, 2010) recorded the high level of cholesterol (75–170 mg/dl) and AP (125.72±11.78) levels in American bison.

The parasitological analysis showed free tigresses from any external or internal parasitic infestations, which could be attributed to the regular use of deworming drugs for circus animals. Although (Lukešová *et al.*, 2000) study the prevalence of parasitic infestations in some circus animals as an indicator of welfare, they found that different parasites were positive, with variant percentages in lions, monkeys, and others. Javaregowda (2016) declared that captive wild carnivores, including tigers and leopards, were held both protozoan parasites and enteric helminths and were positive for one or mixed infection. Moreover, the parasite prevalence rate was 65.3% among carnivores (Barbosa *et al.*, 2020)

CONCLUSION

This research work was studying and evaluating some clinical parameters in three aged South China tigresses who inhabit a local circus in Egypt for the first time to the best of our knowledge. It was found that all the hematological and biochemical blood analyses were within the normal range, except for the presence of anemia. Chloride, cholesterol and blood urea nitrogen levels were high, in addition to a deficiency of total carbon dioxide (tCo2) and platelets. From these results, we conclude that the aging of the tigers is the reason for these changes in blood parameters. Therefore, we recommend giving the necessary vitamins for anemia and medication to reduce cholesterol, then maintaining the vitality of these endangered species. Advanced studies are required to cover the complete picture of tiger status in captivity.

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تقييم الحالة الصحية العامة لبعض أنثى البير في سيرك محلي بالقاهرة , مصر

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أجريت هذه الدراسة لتقييم الحالة الصحية العامة لثلاثة من أنثى البير المتقدمة في السن (٢٠ سنة) والموجودة في سيرك محلي بالقاهرة وذلك للمرة الأولى. يعتبر البير أحد أنواع القطط الكبيرة, والتي تصنف بأنها مهددة بالانقراض. تم تجميع عينات الدم والبراز لأجراء تحاليل الدم والتحاليل البيوكيميائية وفحص الطفيليات. كشفت تحاليل الدم عن وجود نوع من الانيميا مع وجود تغيرات غير معنوية في عدد كرات الدم البيضاء وأنواعها المختلفة. وأظهرت التحاليل البيوكيميائية مستويات طبيعية من انزيمات الكبد , وزيادة نيتروجين الدم , الكرياتينين, ايونات الكلور, والكوليسترول عن المستوى الطبيعي. وفي المقابل, كان هناك نسب طبيعية للسكر, الكالسيوم, الفوسفات, ايونات الصوديوم, ايونات البوتاسيوم. بالإضافة إلى انخفاض نسبة ثنائي أكسيد الكربون وصفائح الدم. الفحص الطفيلي كان سلبيًا وجميع أنثى البير كانت خالية تمامًا من أي طفيليات خارجية أو داخلية. ننصح بعمل دراسات متقدمة لتغطية الصورة الكاملة للحالة الصحية للبير في الأسر.