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Effect of Season, Sex, and Age on Hematological Constituents in Healthy Egyptian Aoudad (*Ammotragus lervia*) in Giza Zoo, Egypt

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ABSTRACT

Egyptian aoudad (*Ammotragus lervia*) is a native antelope in Egypt and is listed as vulnerable according to the IUCN-Red List of threatened species. Little previous research data about the normal hematology of Aoudad are available. Therefore, this study was carried out to determine the normal blood constituents in healthy Egyptian Aoudad kept in Giza Zoo under the effect of season, sex, and age. One hundred and forty animals were used in this study and divided according to seasons into 35 animals in each season, according to sex into 60 females, 45 males, and 35 males in rutting season, and according to age into 50 young and 90 adult animals. The results showed a significant increase ($P<0.05$) of erythrocytes and hemoglobin in winter. Males in the rutting season showed significantly ($P<0.05$) higher levels of erythrocytes ($14.34\pm0.58 \times 10^6$cells/mm^3) than non-rutting males ($12.26\pm0.38 \times 10^6$cells/mm^3) and females ($11.96\pm0.36 \times 10^6$cells/mm^3). The influence of age was clear in erythrocyte count, which increased significantly ($P<0.05$) in young Aoudads. To conclude, this study shows the significant effect of seasons, sex, and age on the blood constituents of Aoudad that should be taken into consideration during the interpretation of laboratory results in healthy and diseased cases.

Key words: Aoudad (*Ammotragus lervia*); Hematological; Rutting; Season; Zoo.

INTRODUCTION

Aoudad (*Ammotragus lervia*) is a native species to north Africa (Cassinello 2015 and Wright 2022). It has six known subspecies according to their geographical distribution and morphological differences: *A. lervia lervia* (Atlas Aoudad), *A. lervia ornate* (Egyptian Aoudad), *A. lervia sahariensis* (Saharan Aoudad), *A. lervia blainei* (Kordofan Aoudad), *A. lervia angusi* (Aïr Aoudad), and *A. lervia fassini* (Libyan Aoudad) (Ansell 1972; Šprem et al. 2020; Derouiche et al. 2020). Egyptian Aoudad (*A. lervia ornate*) is an inhabitant of mountainous regions in Egypt, including Gebel Elba in the South of the Eastern Desert and Gebel Uweinat in the Western Desert (Osborn and Helmy 1980). Egyptian Aoudad has been considered extinct in the wild, and the only existent individuals are found in Egyptian zoos as a part of a national breeding program in captivity (Shackleton 1997). Aoudad has been listed vulnerable by the International Union for Conservation of Nature Red List of Threatened Species, and their population has declined (IUCN 2021).

Sexually mature Aoudads show apparent sexual dimorphism that can be identified through facial and horn morphology (Kavčić 2020). Sexual maturity starts at 14 months in males and 9 months in females. The rutting season mainly occurs in autumn, from September to November (Cassinello 1997). The longevity of Aoudad can exceed 20 years in captivity (Ogren 1965). Studying of Aoudad population in Egypt, especially the captive herd in the Giza Zoo, is a part of the national program for the conservation of Egyptian Aoudad. This survey will provide conclusive data about their normal hematology under the effect of season, sex, and age.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Cairo University (Vet. CU, IACUC), (Approval number, Vet CU 12/10/2021/334; Approval date, October 12, 2021).

One hundred forty clinically healthy Aoudad from the Giza Zoo herd were used to evaluate the complete blood

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count (CBC). Their ages ranged between 3 months and 10 years, which were determined through dentation (Altin 2007; Rahmouni et al. 2019). They were divided according to seasons into 35 in each season, according to sex into 60 females, 45 males, and 35 males in a rut, and according to ages into 50 young and 90 adults. The four seasons were: winter (December to February), spring (March to May), summer (June to August), and autumn (September to November).

Blood samples were collected in EDTA tubes and taken immediately to the lab for examination of CBC, which included erythrocytes, packed cell volume (PCV), total leukocytic count, differential leukocytic count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). They were determined using methods described earlier (Schalm et al. 1975; Peinado et al. 1999; Zapata et al. 2003). Colorimetric estimation of hemoglobin concentration was carried out using Drabkin's solution kit supplied by Spectrum Company, Egypt, and optical density was measured at 540nm by APEL spectrophotometer, Japan. Packed cell volume was determined using heparinized capillary tubes, and then they were centrifugated in a micro-hematocrit centrifuge for 5min at 25,000rpm. The erythrocyte and leukocyte counts were determined using Neubauer's hemocytometer. Fresh blood smears were fixed with methyl alcohol and stained with Diff-Quick III Stain Kit, then used for a differential leukocyte count. Erythrocyte indices were calculated according to the following formula described by Wintrobe (2008): mean corpuscular volume (MCV)= PCV X 10/erythrocyte count; mean corpuscular hemoglobin (MCH)= hemoglobin X 10/ erythrocyte count; mean corpuscular hemoglobin concentration (MCHC)= hemoglobin X 100/PCV.

Statistical analysis

Statistical analysis of the obtained data was carried out by SPSS program version 25. An independent sample t-test was applied for analysis of the data related to age, while one-way ANOVA was used to analyze the data related to sex and seasons. Results were expressed as mean±SE. $P<0.05$ was considered significant statistically.

RESULTS

Table 1 demonstrates the effect of seasons on CBC in Aoudad. In winter, there was a significant ($P<0.05$) increase in hemoglobin, band neutrophils, and monocytes. Erythrocytes showed significantly ($P<0.05$) higher values in winter and autumn, while lymphocytes increased significantly ($P<0.05$) in summer and autumn. MCV showed significantly higher levels ($P<0.05$) in spring, and MCHC showed significantly higher values in autumn ($P<0.05$). Eosinophils significantly increased ($P<0.05$) in winter and spring. However, there was no significance in hematocrit, MCH, total leukocytic count, segmented neutrophils, or basophils under the effect of seasons.

Regarding the effect of sex, Table 2 shows that erythrocytes significantly increased ($P<0.05$) in the rutting males, while the non-rutting males showed a significant rise ($P<0.05$) in MCV and segmented neutrophils. Female Aoudad significantly increased ($P<0.05$) in MCV, MCH, and segmented neutrophils. However, there was no significance in hemoglobin, hematocrit, MCHC, total leukocytic count, band neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Table 3 illustrates the effect of age on CBC in Aoudad and the general mean values of blood constituents. There was a significant increase ($P<0.05$) in erythrocytes and lymphocytes in young Aoudad, while the levels of MCV were significantly higher ($P<0.05$) in the adult Aoudad. However, there was no significance in hemoglobin, hematocrit, MCH, MCHC, total leukocytic count, band neutrophils, segmented neutrophils, monocytes, eosinophils, and basophils. Average hematological constituents in healthy Egyptian aoudad are presented in Table 4.

DISCUSSION

It was suggested that the blood constituents of animals might vary because of many intrinsic influences such as age and sex (Njidda et al. 2013) and extrinsic influences such as climate, nutrition (Birgel 1982), and the number of animals used in the study (Peinado et al. 1999). Concerning the effect of seasons, climatic changes such as fluctuation in temperatures and humidity significantly influenced the hematology in different species (Sothern et al. 1993; Gill et al. 1994).

The values of erythrocytes in the present study ($12.65x10^6/mm^3$) were lower than the values ($15.27x10^6$, $13.2x10^6$, and $13.17x10^6/mm^3$) recorded by Tumbleson (1970), Peinado (1999), and Ferrer (2017), respectively. The higher levels of erythrocytes count in young animals might be attributed to the higher metabolic rates during their growth phase (Meneghini et al. 2016). The decreased total count in older animals can be referred to as the invasion of bone marrow with adipose tissue leading to a reduction in the total number of hematopoietic cells with advancing age (Lima et al. 2015). The higher levels of androgens could explain the influence of sex in rutting males, which enhanced the erythropoiesis process leading to higher erythrocytes count (Leberbauer et al. 2005).

The results of Hb in this study (13.97g/dL) were higher than Peinado's (1999) (12.9g/dL), while it was lower than Tumbleson's (1970) and Ferrer's (2017) (14.3 and 14.02g/dL), respectively. The average packed cell volume (PCV) in this study (38.62%) was less than the averages (42.20 and 40.6%) provided by Ferrer (2017) and Tumbleson (1970), respectively. However, it was more than the average (33.3%) provided by Peinado (1999).

In this research, the Mean Corpuscular Volume (MCV) values (32.11fL) were nearly similar to Ferrer's (2017) (32.38fL), while it was higher than Tumbleson's (1970) (27.3fL) and Peinado's (1999) (25.2fL). The values of Mean Corpuscular Hemoglobin (MCH) (10.72, 9.79, and 9.6pg/cell) obtained by Ferrer (2017), Peinado (1999), and Tumbleson (1970), respectively, were lower than what was reported in this study (11.56pg/cell). On the other hand, the Mean Corpuscular Hemoglobin Concentration (MCHC) result in this work (36.47%) was higher than the results provided by Tumbleson (1970) (35.2%) and Ferrer (2017) (33.18%), while it was lower than Peinado's (1999) (39.1%).

Table 1: The effect of season on the hematological constituents in healthy Egyptian aoudad

Parameter	Unit	Winter	Spring	Summer	Autumn
Erythrocytes	x10^{6}cells/mm^3	13.80±0.63a	11.16±0.39b	12.19±0.37ab	13.49±0.51a
Hb	g/dL	14.45±0.34a	13.22±0.34b	13.96±0.21ab	14.26±0.31ab
Hematocrit	%	39.35±0.97	39.67±0.75	38.88±0.95	36.62±0.75
MCV	μm^3	30.58±1.63bc	36.68±1.31a	33.14±1.41ab	28.06±0.95c
MCH	pg/cell	11.24±0.61	12.18± 0.41	11.83±0.41	10.96±0.39
MCHC	%	36.90±0.72ab	33.59±0.72c	36.33±0.76b	38.98±0.49a
Total leukocytic count	x10^{3}cells/mm^3	11.27±0.77	9.74±0.43	11.22±0.68	11.04±0.70
Relative band neutrophils	%	3.23±0.55a	1.77±0.26b	1.14±0.35b	1.54±0.33b
Absolute band neutrophils	x10^{3}cells/mm^3	0.40±0.07a	0.14±0.02b	0.12±0.04b	0.18±0.04b
Relative segmented neutrophils	%	58.25±1.71	67.02±1.61	59.71±1.96	57.08±2.18
Absolute segmented neutrophils	x10^{3}cells/mm^3	6.59±0.52	6.51±0.32	6.62±0.37	6.34±0.46
Relative lymphocytes	%	29.47±1.18bc	26.11±1.63c	34.28±2.02ab	38.45±2.14a
Absolute lymphocytes	x10^{3}cells/mm^3	3.29±0.23ab	2.59±0.23b	4.07±0.41a	4.21±0.37a
Relative monocytes	%	5.70±0.50a	2.80±0.50b	2.62±0.58b	2±0.57b
Absolute monocytes	x10^{3}cells/mm^3	0.62±0.07a	0.25±0.04b	0.31±0.08b	0.20±0.05b
Relative eosinophil	%	2.5±0.47a	2.11±0.32a	1.54±0.28ab	0.74±0.24b
Absolute eosinophils	x10^{3}cells/mm^3	0.24±0.05a	0.20±0.03a	0.16±0.03ab	0.06±0.02b
Relative basophils	%	0.47±0.17	0.34±0.15	0.34±0.15	0.17±0.12
Absolute basophils	x10^{3}cells/mm^3	0.04±0.02	0.02±0.01	0.04±0.01	0.02±0.01

Values (mean±SE) within the same row having different letters are significantly (P<0.05) different.

Table 2: The effect of sex on the hematological constituents in healthy Egyptian aoudad

Parameter	Unit	Non-rutting male	Rutting male	Female
Erythrocytes	x10^{6}cells/mm^3	12.26±0.38b	14.34±0.58a	11.96±0.36b
Hb	g/dL	13.69±0.26	14.28±0.34	14.01±0.24
Hematocrit	%	38.86±0.73	38.34±1	38.62±0.66
MCV	μm^3	32.97±1.29a	27.72±1.02b	34.03±1.15a
MCH	pg/cell	11.52±0.36ab	10.39±0.41b	12.29±0.39a
MCHC	%	35.69±0.72	37.38±0.60	36.52±0.60
Leukocytes	x10^{3}cells/mm^3	11.40±0.56	9.97±0.71	10.89±0.50
Relative band neutrophils	%	1.91±0.33	2±0.47	1.86±0.31
Absolute band neutrophils	x10^{3}cells/mm^3	0.22±0.04	0.23±0.06	0.20±0.04
Relative segmented neutrophils	%	63.64±1.70a	54.11±1.84b	62±1.43a
Absolute segmented neutrophils	x10^{3}cells/mm^3	7.18±0.38a	5.35±0.41b	6.70±0.30a
Relative lymphocytes	%	29.29±1.68b	38.46±2a	30.47±1.31b
Absolute lymphocytes	x10^{3}cells/mm^3	3.40±0.29	3.88±0.38	3.46±0.26
Relative monocytes	%	3.20±0.53	3.54±0.64	3.15±0.42
Absolute monocytes	x10^{3}cells/mm^3	0.37±0.08	0.32±0.06	0.35±0.05
Relative eosinophils	%	1.73±0.29	1.54±0.36	1.81±0.29
Absolute eosinophils	x10^{3}cells/mm^3	0.18±0.03	0.13±0.03	0.19±0.03
Relative basophils	%	0.36±0.13	0.29±0.15	0.34±0.12
Absolute basophils	x10^{3}cells/mm^3	0.04±0.01	0.03±0.02	0.04±0.01

Values (mean±SE) within the same row having different letters are significantly (P<0.05) different.

Table 3: The effect of age on the hematological constituents in healthy Egyptian aoudad.

Parameter	Unit	Young	Adult
Erythrocytes	x10^{6}cells/mm^3	13.43±0.43*	12.23±0.32
Hb	g/dL	14.28±0.29	13.81±0.18
Hematocrit	%	38.28±0.74	38.78±0.55
MCV	μm^3	29.73±1.08	33.44±0.93*
MCH	pg/cell	11.04±0.36	11.86±0.31
MCHC	%	37.37±0.48	35.96±0.52
Leukocytes	x10^{3}cells/mm^3	11.47±0.57	10.51±0.41
Relative band neutrophils	%	2.32±0.42	1.68±0.21
Absolute band neutrophils	x10^{3}cells/mm^3	0.28±0.05	0.18±0.03
Relative segmented neutrophils	%	54.2±1.78	64.02±1
Absolute segmented neutrophils	x10^{3}cells/mm^3	6.12±0.33	6.72±0.27
Relative lymphocytes	%	37.92±1.72*	28.86±1.03
Absolute lymphocytes	x10^{3}cells/mm^3	4.44±0.32*	3.04±0.18
Relative monocytes	%	3.32±0.59	3.24±0.32
Absolute monocytes	x10^{3}cells/mm^3	0.38±0.08	0.33±0.04
Relative eosinophils	%	1.68±0.33	1.74±0.21
Absolute eosinophils	x10^{3}cells/mm^3	0.17±0.03	0.17±0.02
Relative basophils	%	0.52±0.16	0.22±0.07
Absolute basophils	x10^{3}cells/mm^3	0.06±0.02	0.02±0.01

Asterisk in a row shows significant (P<0.05) difference between young and adult animals.

Table 4: Average hematological constituents in healthy Egyptian aoudad

Parameters	Unit	Mean±SE
Erythrocytes	x10^6cells/mm^3	12.65±0.25
Hemoglobin	g/dL	13.97±0.15
Hematocrit	%	38.62±0.44
MCV	μm^3	32.11±0.72
MCH	pg/cell	11.56±0.23
MCHC	%	36.47±0.37
Leukocytes	x10^3cells/mm^3	10.82±0.33
Rel. band neutrophils	%	1.91±0.20
Abs. band neutrophils	x10^3cells/mm^3	0.21±0.02
Rel. segmented neutrophils	%	60.54±0.98
Abs. segmented neutrophils	x10^3cells/mm^3	6.52±0.21
Rel. lymphocytes	%	32.10±0.97
Abs. lymphocytes	x10^3cells/mm^3	3.54±0.17
Rel. monocytes	%	3.26±0.29
Abs. monocytes	x10^3cells/mm^3	0.35±0.03
Rel. eosinophils	%	1.72±0.17
Abs. eosinophils	x10^3cells/mm^3	0.17±0.02
Rel. basophils	%	0.33±0.07
Abs. basophils	x10^3cells/mm^3	0.03±0.01

Rel.=Relative; Abs.=Absolute.

The total average leukocytic count reported in this research (10.82x10^3 cells/mm^3) was higher than Peinado's (1999) (3.70x10^3 cells/mm^3) and Ferrer's (2017) (9.92x10^3 cells/mm^3) while it was lower than Tumbleson's (1970) (11.2x10^3 cells/mm^3). The band neutrophils' general mean in this study was (0.21± 0.02x10^3 cells/mm^3), higher than the value which was reported by Tumbleson (1970) (0x10^3 cells/mm^3), while it was lower than Ferrer's (2017) (0.33x10^3 cells/mm^3). The segmented neutrophil values obtained in this study were (6.52x10^3 cells/mm^3) in agreement with Ferrer's (2017) (6.50x10^3 cells/mm^3). In contrast, they were lower than the values reported by Tumbleson (1970) (7.4x10^3 cells/mm^3) and higher than Peinado's (1999) (1.91x10^3 cells/mm^3).

The lymphocyte values of Peinado (1999) (1.62x10^3 cells/mm^3) and Ferrer (2017) (2.65x10^3 cells/mm^3) were lower than the value obtained in this work (3.54x10^3 cells/mm^3) which agreed with Tumbleson (1970) (3.4x10^3 cells/mm^3). The monocyte values in this research (0.35x10^3 cells/mm^3) were higher than the values (0.2x103, 0.05x10^3, and 0.12x10^3 cells/mm^3) reported by Tumbleson (1970), Peinado (1999) and Ferrer (2017), respectively.

The eosinophils documented by Tumbleson (1970) (0.1x10^3 cells/mm^3) and Peinado (1999) (0.16x10^3 cells/mm^3) were approximately similar to the values obtained in this study (0.17x10^3 cells/mm^3) and lower than Ferrer's (2017) (0.30x10^3 cells/mm^3). The increased number of eosinophils in winter and spring could be attributed to the exposure of animals to seasonal allergens such as pollen grains.

In the current study, basophils were 0.03x10^3 cells/mm^3, which were lower than the values reported by Tumbleson (1970) (0.1x10^3 cells/mm^3), while Peinado (1999) and Ferrer (2017) reported them as 0x10^3 cells/mm^3.

Conclusion

It is concluded that seasons significantly affect blood constituents, followed by sex, while age has a mild effect. The results provided in this study can be considered a reference for the hematology of *Ammotragus lervia* in Egyptian zoos, which veterinarians can use to interpret the laboratory results of healthy and diseased cases. Further investigations are required to evaluate the overall health status.

Conflict of Interest

The authors declare no conflict of interest. In addition, none of the authors has any financial or personal relationships that can inappropriately influence or bias the paper's content.

Authors' contributions

All authors contributed to the study's conception and design. Soheir Aasem Abd Al-Galeel performed animal treatment, sample collection and analysis, and data analysis, as well as writing the first draft of the manuscript. Taher Ahmad Baraka, Sabry Ahmed Mousa, and Mohamed Ragaii Younis made the design of the experiment and edited the manuscript. All authors read and approved the final manuscript.

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