HEMATOLOGICAL AND PARASITOLOGICAL INVESTIGATION IN *TESTUDO GRAECA* (LINNAEUS,1758) TORTOISE KEPT IN CAPTIVITY IN EGYPT

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ABSTRACT

Chelonians (Turtle and Tortoise) are kept in captivity as pet animals. Testudo. graeca called Spur thighed tortoise for its large conical tubercle present on each Thigh. T. graeca considered endangered across its entire range. They were illegally imported from Libya to Egypt. The aim of this work is to determine normal Hematological parameters and gastrointestinal parasite in Spur-thighed tortoise. Blood samples were taken from 37 tortoises, 13 female and 24 Male. Fecal samples were collected from 33 tortoises. Feces were examined by qualitative and quantitative methods. RBCs Count, WBCs Count, PCV, HB, MCV, MCH, MCHC value and DLC% were measured. Strongylid and Oxyurid eggs founded. Mean RBCs count in tortoise was 0.68 10^6 /µL, Hemoglobin (Hb) concentration was 8.26 g/dl, Packed cell Volume (PCV) was 25.12%. Lymphocytes % were 30.3 %. Monocytes (%) were 25.03. Heterophils (%) were 40.44%. Eosinophils (%) were 5.87%. Basophils (%) were 0.05%.We report hematological parameters in normal and parasitic infection. In

addition, we investigate parasites in Spur-thighed tortoise.

INTRODUCTION

Testudo.graeca (T.graeca) is one highly traded species ***(Theile, S. 2002)***. *T.graeca* complex involve 37 % of all traded *Testudo* ***(Turkozan et al.,2008)***. *T.graeca* is widely spread in Western and Southern Europe, Southwest Asia ***(Uetz, P. and Hošek, J., 2014)***. *T. graeca* is considered endangered across its entire range (classified as vulnerable; IUCN, 2002). It is considered a vulnerable species in Europe ***(COX & TEMPLE, 2009)***. *Testudo. graeca* are small tortoises. There are tubercles on their thighs, either side of the tail. *T.graeca* also called Spur thighed tortoise for its large Conical tubercle present on each Thigh. ***(Mcarthur et al., 2004; Uetz, P. and Hošek, J., 2014).***

Blood parameters used to determine the physiologic status of chelonian. It considered a mean in diagnosis of chelonian diseases ***(Oliveira-Junior et al., 2009)***. Many factors affect in Chelonian blood parameters such as season, age, sex, geographic sites, reproductive and health status ***(Jacobson, E. R., 2007)***. There are significant differences in some blood parameters between species in the same *Testudo* species. ***(Mathes, K. et al., 2006).***

Determining the significance of a parasite can be difficult. Parasitic infections are often chronic. It does not give clear clinical symptoms in optimal habitat and maintenance conditions. Susceptibility to disease caused by parasitism is related to stress, environmental temperature, hygiene, concurrent disease, the number of parasites, availability of intermediate host, nutritional status and age. This can supress the

immune system causing clinical form of parasitic diseases ***(Diaz-Figueroa, O., 2005 ;Rataj et al., 2011)***.

This study aimed to evaluate normal hematological parameters in male and Female Spur- thighed tortoise (*T.graeca*), investigate their gastrointestinal parasites and determine relation between fecal egg count and Hematological parameters.

MATERIALS AND METHODS

1-Animal:

Thirty seven Tortoises, 13 female and 24 Male kept in Captivity. These tortoises were smuggled from Libya by pet animal shops. They were collected from pet animal shops in Cairo, Egypt. They were weighed. Total straight carapace length, total straight carapace width and Plastron length were measured according to ***(Barrows, M., 2004)***. Their sex was determined according to ***(Mcarthur et al., 2004)***. They were fed green foods (lettuce and cucumber) throughout the study. The animals were examined clinically for presence any nasal or ocular discharge, ectoparasite and appetite.

2-Sample:

Samples were collected during the period from February to August, 2018.

A) Blood Sample:

While tortoise was physically restrained. Blood Sample was collected from post occipital venous plexus. Blood sample were collected according to ***(Lloyd, M. and Morris, P., 1999)***. The blood was drawn with 25 gauge heparinized needle attached to 3 ml syringe. It poured

gently in heparinized vacutainer tube for hematological parameter determination ***(Tavares-Dias et al., 2008)***.

B) Fecal Sample:

A total number of fecal samples were 32 samples. Reptiles usually defecate during physical examination. Feces can be collected by gentle cloacal manipulation ***(Hernandez-Divers,S.J. and Cooper,J.E. ,2004)***. The animal kept with clean underground paper and the feces collected while it is still fresh. ***(Mader,D.R. ,2006).***

Fecal sample were kept in plastic bags. The samples were refrigerated at 4 ˚C or conserved by formalin 10 % until the Coprological examinations were carried out within 24 h from sampling ***(Traversa D., 2005)***.

These samples Transferred to the Laboratory of Animal medicine department at Faculty of Veterinary medicine, South Valley University in Qena. Samples were examined by qualitative method, while only twenty (20) samples were examined by quantitative Mc-Master method.

3- Hematological Tests:

RBCs and WBCs counts were determined by the ***Natt and Herrick (1952)*** method using a Newbauer hemocytometer. Hemoglobin concentration measured By colorimetric endpoint cyanomethemoglobin method using Drabkin solution. Packed cell Volume (PCV) was determined by microhematocrit centrifugation technique ***(Jain, 1986)***. MCV, MCH and MCHC were calculated mathematically according to ***(Coles, 1986)***.

A blood smear were made and stained with Giemsa stain for Differential Leucocytic count ***(Coles,1986;Chung,C.,2009)***.

4- Fecal Examination:

Direct smear was performed as describe by ***(Urquhart et al, 2001)***. Fecal Flotation method was performed according to ***(Cable et al, 1985)***. Sedimentation method made according to ***(Bowman,D.D.,2009)***. Mc-Master quantitative method was achieved according to ***(Abdel-Rahman et al.,1982)***.

5- Statistical analysis:

All values were analyzed for statistically significant difference ($P < 0.05$) by a two-sample t-test assuming unequal variance. In addition, significant differences between cumulative means of the three size classes were identified by ANOVA and analyzed for significant difference ($P < 0.05$). These tests were made using Graphpad Prism 8 program.

RESULTS

Clinical examination of tortoises revealed that clinical signs appeared on tortoise except for 3 tortoises appeared anorexia, lethargy, and weakness. Morphometric measurements were recorded in Table 1.

Table (1): Morphometeric measurements of Spur-thighed tortoises

Measurement Sex	Male		Female	
	Mean±SD	Min-Max	Mean±SD	Min-Max
Weight (gm)	362.68±114.7	200-650	354.6±228.02	100-800
Total straight Carapace Length (cm)	12.5±1.3	10.5-15.5	11.7±2.5	8-16

Total straight Carapace width (cm)	10.34±1.5	8.5-13	10.2±2.3	6.5-13.5
Total curved Carapace width(cm)	15±1.4	12.5-18	14.8±3.3	9.5-19.5
Plastron Length(cm)	10.2±1.1	8.5-13	10.1±2.1	7-13

Hematological parameters were compared between male and female *T.graeca*. These parameters founded in Table 2.

In relation between egg count and Hematological parameters, Results founded in Table 4. Differential leucocytic count (DLC) was performed in Table 5,figure 2. In relation between egg count and DLC, Results founded in Table 5.

Table (2): Hematological parameters for male and Female *testudo.graeca* (** highly significant difference from female at $p<0.01$)

Parameters Sex	**Male**		**Female**	
	Mean± SD	**Min-Max**	**Mean± SD**	**Min-Max**
RBCs Count(^{106}/µl)	0.78±0.20**	0.5-1.24	0.49±0.14	0.34-0.7
Hemoglobin (g/dl)	8.16±2.23	5.3-13.4	7.42±2.42	4.2-11.9
Hematocrit (%)	27.61±5.8**	18-37	20.25±4.50	15-27
MCV (fl)	347.65±74.02	222.9-485.3	385.61±71.1	314.2-523.8
MCH (pg)	118.03±47.35	56.6-223.6	135.78±39.3	87.1-182.3
MCHC (g/dl)	34.1±8.9	22-51.1	36.11±.11.84	23-54
WBCs count(10^{3}/µl)	3.99±2.18	1-8	3.5±1.27	1.5-4.5

Twenty tortoises from 32 founded positive for *Strongylid* species eggs figure 1 with percent 62.5 %. *Oxyurid* species eggs figure 1 founded in 11 tortoises from 32 with percent 34.4%. Egg count per gram recorded. Average Number of eggs per gram recorded in Table 3. There was no significant increase in male egg count than female.

Fig. (1): Flotation method show: A- Show *Strongylid* egg. B- Show *Strongylid* egg (vertical arrow) and *Oxyurid* egg (horizontal arrow)

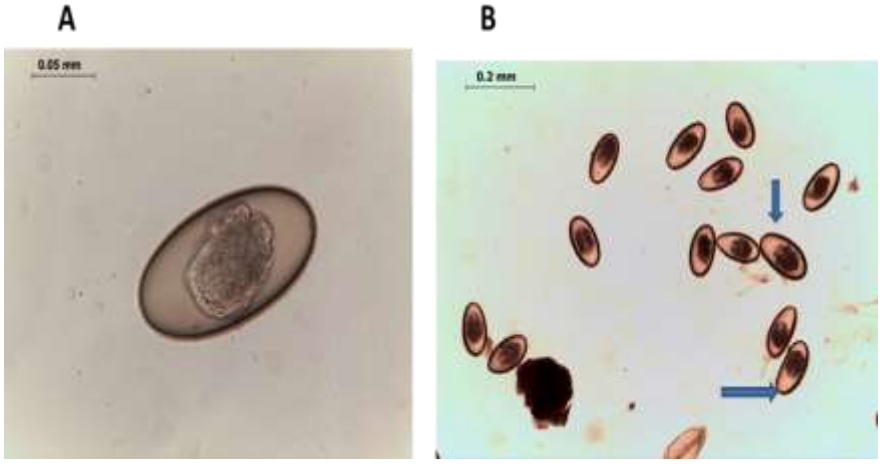

Table (3): Quantitative Mc-Master result in *T.graeca* (egg/ gram)

Animal	Mean ±SD	Min-Max
Male	3276.9±3778	800-14800
Female	7516.66±6949	1200-16800

Table (4): Relation between Hematological Parameters of *Testudo.graeca* and Fecal egg count (egg/gram) (* significant difference from mean value of apparently healthy at $p<0.05$)

Parameters	Values of *T.graeca* (apparently healthy)	Higher degree (>3000 egg per gram)	moderate degree (<3000 egg per gram)

	Mean± SD	Min-Max	Mean± SD	Min-Max	Mean± SD	Min-Max
RBCs Count(10^6/µl)	0.68±0.23	0.34-1.24	0.50±0.09*	0.39-0.65	0.68±0.16	0.31-0.89
Hemoglobin (g/dl)	8.26±2.5	4.2-13.4	6. 2±1.62*	4.2-8	7.66±1.99	5.3-10.2
Hematocrit (%)	25.12±6.40	15-37	20±6.78	15-30	25.6±6.5	18-33
MCV (fl)	355.6±74.1	22.9-523.8	383.5±56.53	327.2-461.5	362.72±89.9	266.6-485.3
MCH (pg)	115.3±42	56.6-223.6	120.4±53.2	65.9-223.6	101.7±20	77.9-136.2
MCHC (g/dl)	34.3±8.22	22-51	32.82±5.44	26.6-38.7	32.01±5.4	26.5-39.3
WBCs count(10^3/µl)	3.68±1.9	1-8	3.39±1.3	2-5.5	4.7±2.04	1.75-7.25

Fig. (2): Leucocyte and Erythrocyte cells in *Testudo.graeca*. A and B show Monocyte, C show Lymphocyte , D show Erythrocyte, E show Eosinophile, F show heterophile.

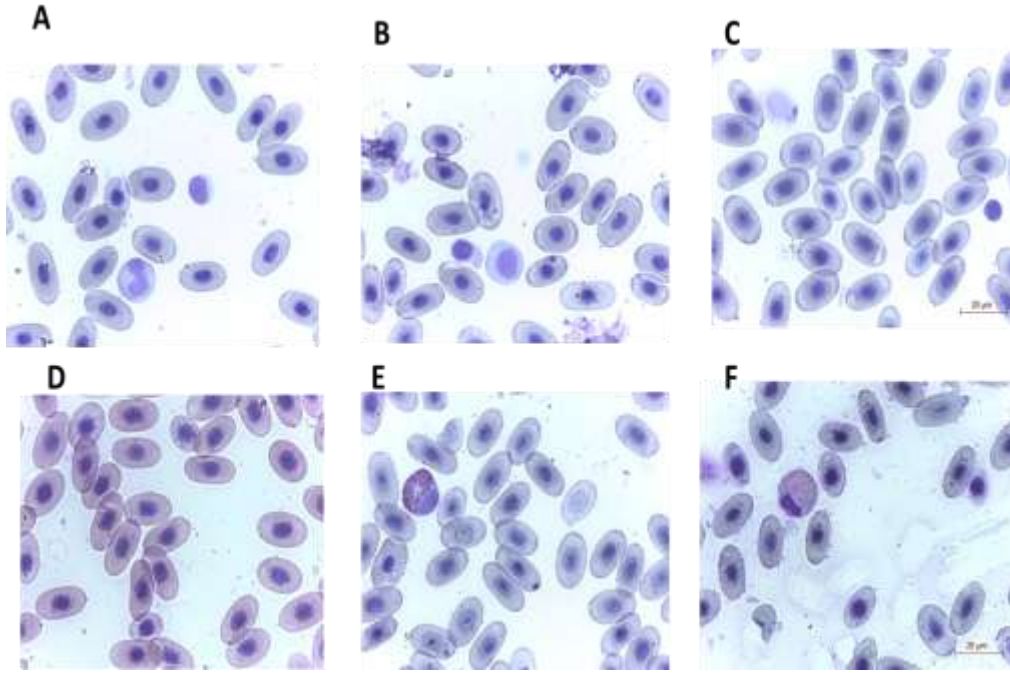

Table (5): Relation between Differential Leucocytic count (DLC) and fecal egg count in *Testudo.graeca* (* significant difference from apparently healthy at p<0.05)

Parameters	Values of *T.graeca*	Higher degree	moderate degree

	(apparently healthy)		(>3000 egg per gram)		(<3000 egg per gram)	
	Mean± SD	**Min-Max**	**Mean± SD**	**Min-Max**	**Mean± SD**	**Min-Max**
Lymphocytes (%)	30.3±6.85	14-41	34.2±7.85	23-41	29.3±2.9	25-34
Monocytes (%)	25.03±7.8	13-42	21.2±9.7	14-42	32.4±3.3*	28-36
Heterophils (%)	40.44±11.72	21-65	42.83±12.73	25-62	31.3±6.75*	21-43
Eosinophils (%)	5.87±4.5	1-15	9±5.56	4-15	8.2±4.08	4-13
Basophils (%)	0.05±0.2	0-1	0.25±0.46	0-1	0	0
Plasma cell (%)	0.8±2	0-9	0.375±0.74	0-2	1.2±2.78	0-9

DISCUSSION

Hematological parameters of apparently healthy *T.graeca* tortoise were determined. RBCs of *T.graeca* were oval nucleated cells. The nucleus was oval centrally located. These results agreed with ***(Tosunoglu, M. et al., 2005)***. There was a highly significant increase in RBCs count in male than female. These results agreed with ***(Hidalgo-Vila, J. et al., 2007;Hamooda, E.A.F et al., 2014)***.

There was no significant increase in male than female Hemoglobin concentration. These results agreed with ***(Lewbart, G.A. et al., 2018)***. There was a highly significant increase in male PCV than female. These result agree with ***(Hamooda, E.A.F et al., 2014; Andrean, G et al., 2014;Lo´pez,J et al., 2017)*** who recorded PCV in *T. graeca, Testudo hermanni* and Ploughshare tortoises respectively.

Mean of (MCV), (MCH) and (MCHC) was calculated and appeared no significant differences between male and Female. These results agreed with ***(Christopher, M. et al., 1999)***.

Differential Leucocytic count Results in Spur thighed tortoise (*T.graeca*) nearly agreed with ***(Tosunglu, M. et al., 2005)*** and disagreed

with ***(Hidalgo-Vila, J. et al.,2007)*** in Free-Living Mediterranean Pond Turtles. This variation in results attributed to differences between and within species. These differences have been attributed to nutrition, sex, age, population dynamics, environmental conditions and puncture site ***(Frye, F. L., 1991; Stacy, B.A. and Whitaker, N., 2000; Bonnet, X. et al, 2016)***.

Fecal examination was performed .*Oxyurid* species eggs and *Strongylid* species eggs founded. These results agreed with ***(Lichtenfels and Stewart,1981;Shanker,R. et al., 2015)*** who described *Strongyloidea* and *Oxyuroidea* from the large intestines of eight gopher tortoises from Georgia. Traversa D., 2005 isolated oxyurid eggs in parasitized tortoise.

Percent of *Strongylid* was 62.5 % and *Oxyurid* egg was 34.4 %. *Oxyurid* % agreed with ***(Rataj, A.V. et al., 2011)*** who stated that Oxyurid nematoda (*Pharyngodonidae, Tachygonetria* sp.) founded in 33.3% to 92.5%of tortoises. This result disagreed with ***(Satorhelyi T. and Sreter T.,1993)*** who reported that prevalence of oxyurid egg was 69 %. Percent of *Strongylid* was nearly agree with ***(Diaz-Figueroa, O., 2005)*** who reported that Stongyloidae prevalence was 50 % in Louisiana Gopher Tortoise (*Gopherus polyphemus*).

In relation between egg count and Hematological parameters, These results agreed with ***(Millan,J. M. et al., 1997; Stacy, B.A. and Whitaker, N. 2000)*** who stated that variable endoparasite burden could account for lower PCV and RBC in examined populations of *Crocodylus. palustris.* In addition, ***(Diaz-Figueroa, O., 2005)*** stated that nematodiasis can cause anorexia and anemia.

In relation between Differential Leucocytic count (DLC) and Egg count, There was no significant increase in Eosinophile. These results

agreed with ***(Campbell, T. W., 1996; Wilkinson, R.,2003; Chitty J. and Raftery A., 2013)***. There was a significant increase in Monocyte in moderate egg count. This result agreed with ***(Veiga et al., 1998; Bonadiman et al., 2010)*** who reported a marked increase in Monocyte in *Psammodromus algirus* lizards with *Ixodes ricinus and Ameiva ameiva* lizards that were infected with Hemolivia. Monocytosis occured in chronic infection/ inflammatory condition***(Chitty J.and Raftery A.,2013)***.

In relation to egg count, There was a significant decrease in Heterophile in moderate egg count. This result agreed with ***(Chitty J. and Raftery A., 2013)*** who reported that heterophile decrease in inflammatory conditions where the animal is in poor condition.

CONCLUSION

Strongylid and *Oxyurid* eggs were founded in fecal examination. We report differences in some Hematological parameters in both male and female Spur thighed tortoises and in their relation to parasite Infection.

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