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Hemato-Biochemical changes in dogs infected with *toxocara canis* in Hurghada and Luxor governorate

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Abstract

Toxocara canis is a gastrointestinal nematode affecting dogs with significant public health importance. this study is conducted to find the hematological and biochemical changes in dogs infected with *toxocara canis* and to detect the effects of sex, age, and season on the percent of infection with this parasite. A total of 70 dogs were included in this experiment. Fecal, hematologic and serum samples were collected from veterinary clinics in Hurghada and Luxor governorate. Microscopic analysis of fecal samples revealed three types of intestinal parasites: *Toxocara canis* (n= 19), *Toxascaris leonina* (n= 5), *Isospora canis* (n=4) and mixed infection of *Toxocara canis* and *Isospora canis* (n= 3). Whole blood samples were examined for total erythrocytic count, hemoglobin, packed cell volume, total leukocytic count and differential leukocytic count. Serum samples were separated and analyzed for total protein, albumin, Alanine aminotransferase and Aspartate aminotransferase. Significant decrease in red blood cells count, hemoglobin, and packed cell volume along with significant increase in white blood cells count and eosinophils were recorded in dogs infected with *Toxacara canis*. Significant decrease in total protein, albumin accompanied by significant increase in Alanine aminotransferase, Aspartate aminotransferase values were found. Sex, season, and breed showed non-significant differences in infection percent of intestinal parasites, but age had high impact on infection percent of intestinal parasites.

Keywords:

Aspartate aminotransferase, Dogs, Hemoglobin, Total protein, Toxocara.

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Introduction

Dogs perform a variety of cultural, social, and economic functions in the society. They can be kept as pets and companion animals, for hunting, as guards, draught animals, for food, or for commercial purposes (Savolainen et al., 2002).

Dog parasites exist internally and externally, wide range of them are specific and live only on dogs. Infestation with gastrointestinal parasites occur in all ages of dogs with the greatest incidence in puppies (Seah et al., 1975). Gastrointestinal helminths of dogs have serious impact on the host and human beings. It can cause several losses manifested by inhibition of immune response to infectious diseases, retardation of growth, lowered work and feed efficiency and general ill- health. Animals affected with parasites manifest a variety of signs depending on the parasite burden and species (Soulsby, 1982). *Toxocara canis* is one of the most common and worldwide prevalent parasites that infects canids which act as definitive hosts for this parasite (Bugg et al., 1999; Robertson and Thompson, 2002). Eggs of *T. canis* are Sticky, thick-shelled eggs can survive harsh environmental conditions (Daryani et al., 2009). infestation occurs by ingestion of eggs containing infective stage larvae; transplacental infection and trans-mammary route are another way for transmission of *T. canis* infection (Overgaauw, 1997). When the larva passes on to the alimentary tract, pups suffer from vomiting, diarrhea, distended abdomen, emaciation, poor coat quality and, pain which can be represented by whine, shriek of puppies and, overlapping hind limbs while standing or walking. In adult dogs, the infestation is usually subclinical and there are no respiratory manifestations. Sings restricted to diarrhea, weight loss, and general malaise (Epe, 2009). Intestinal helminths are found to have deleterious effects on blood parameters and cause microcytic hypochromic anemia in parasitized animals (Qadir et al., 2011). (Kumar et al., 2014; Salem et al., 2015) recorded a significant reduction in RBCs count, hemoglobin, and PCV levels while, leukocytosis with significant eosinophilia and neutrophilia has been observed in dogs infected with *T. canis*.

The incidence of parasites in dogs can be related to many factors such as age, gender, and breed which are considered the most common ones, seasonal variations and diversity indexes also can be included (Oliveira-Sequeira et al., 2002; Eguı́a Aguilar et al., 2005).

This study aimed at determining the levels of some hematological and biochemical parameters on the *T. canis* infected and clinically healthy dogs and compared the data acquired.

Materials and methods

1. Ethical statement:

All animals included in this study were handled according to the regulations of the Animal Ethics Committee at the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt, with good animal practice following the guidelines of The Research Code of Ethics (RCOE-SVU) at the South Valley University.

2. Animals

Seventy dogs of both sexes and different ages (Age =1 month to 4 year) and breeds (local and exotic) from animal care hospital in Luxor governorate and Hurghada were examined for intestinal nematodes during the period from May 2019 to November 2020.

Out of 70 dogs, 39 animals were used as a healthy control group after thorough clinical and laboratory examination, Microscopical fecal examinations were done three times on three consecutive days to be sure free from intestinal parasite.

3. Samples

A) Blood samples:

Two blood samples were collected from the cephalic vein, whole blood sample for hematological studies and a clear non-hemolysed serum sample was prepared for biochemical studies (Jain, 1986)

B) Fecal samples:

A total number of 70 fecal samples were collected from dogs in Luxor governorate and Hurghada according to (Charles and Hendrix, 1998).

4. Clinical examination:

Clinical examination of dogs was carried according to (Englar, 2017). The clinical symptoms were recorded, with special focus on presence of diarrhea, abdominal pain, nausea/vomiting and itching in perianal region, body condition, and mucous membrane examination.

5. Fecal examination:

Fecal samples were examined macroscopically for color, blood, mucous, consistency and presence of gross parasites. Microscopic examination was performed using direct smear (Urquhart et al.,2001).

Fecal floatation method was performed according to (Cable et al.,1985). Sedimentation technique was performed according to (Abdel-Rahman et al.,1982).

6. Hematological examinations:

Red blood cells (RBCs) and white blood cells (WBCs) counts were determined according to manufacture instruction using a (NEUBAUER HEMOCYTOMETER, Germany).

Hemoglobin (Hb) concentration measured by colorimetric endpoint cyanomethemoglobin method using DRABKIN SOLUTION. Packed cell volume (PCV) was determined by the microhematocrit centrifugation technique (Jain, 1986). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated mathematically according to (Coles, 1986).

Serum samples were analyzed for total protein (TP), Albumin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), urea, and creatinine spectrophotometrically (UV-1201, Shimadzu, Japan) using commercial biochemical kits (SPECTRUM DIAGNOSTICS, Egypt) and according to the manufacture instruction.

7. Statistical analysis:

The data analysis was performed using of Excel spreadsheet (Microsoft Excel 2016) for descriptive statistics. Data were presented as Mean ± SD. Significant of difference between health and diseased group was determined by T-test using the PRISM statistical software (Graph Pad Prism 7 program).

Results

Out of 70 examined dogs, 48 dogs were apparently healthy showed no clinical signs (asymptomatic) and 22 dogs showed signs of disease (symptomatic). Symptomatic dogs were presented with one or more of the following signs: emaciation, off food, diarrhea, abdominal pain, dull hair coat, hair shedding, pale mucous membrane, and vomiting (Table 1).

Fecal examination of both symptomatically and asymptomatically dogs revealed 31 dogs were infested with nematodes and protozoa (22 symptomatic and 9 asymptomatic) (Table 2).

Table 1: Clinical symptoms observed in dogs infested with parasites.

Groups	Group 1	Group 2	Group 3	Group 4
Spp. infection	*Toxocara canis*	*Toxascaris leonina*	Cystoisospora spp.	**Mixed infection** (*Toxocara canis* with *Isospora canis*)
Diarrhea	+	+	+	+
Abdominal pain	+	-	+	+
Nausea/vomiting	+	-	-	-
Itching in perianal region	-	-	-	-
Body condition	Poor	Poor	Poor	Poor
Mucous membrane	Pale	Pale	Pale	Pale

Table 2: Infection percentage of nematodes in symptomatic and asymptomatic examined dogs.

Parasite	Symptomatic (n=22) Positive (n=22)	Asymptomatic (n=48) Positive (n=9)
Toxocara canis	12 (54.5%)	7 (14.6%)
Toxascaris leonina	4 (18.2%)	1 (2.1%)
Isospora canis	3 (13.6%)	1 (2.1%)
Mixed Infection	3 (13.6%)	0 (0.0 %)
Total Infection/ percentage (%)	22 / (100%)	9 / (18.8%)
Total Infection/ percentage (%)	0 (0.0%)	39 (81.3%)

Fecal examination:

Macroscopic examination of the feces showed no gross parasites. Microscopic examination of the feces revealed presence of eggs of *Toxocara canis* (Fig.1-A), *Toxascaris leonina* (Fig.1-B) and *isospora* oocyst (Fig.1-C).

The study revealed the overall percentage of intestinal parasites infestation was found to be 44.3% (31/70). The infection percent was 65.5% (19/29) in puppies and 36.8% (7/19) in dogs (from 3-6 months of age) and 22.7% (5/22) in adult dogs (above 6 months of age). Furthermore, the overall percentage of infection was 44.7% (17/38) in male dogs and 43.8% (14/32) in females (Table 4). Three types of GI parasites were detected: the predominant one was *Toxocara canis* (27.1%) followed by *Toxascaris leonina* (7.1%) then *isospora canis* (5.7%). three of examined dogs (4.3%) had been infected with more than one parasite (*Toxocara canis* and *isospora canis*) (Table 3).

Factors affecting the infestation with intestinal nematodes:

Age, season, sex, and breed were the factors affecting infection percent of intestinal parasites.

According to age, puppies from 1-3 months showed higher significant infection percent ($P<0.01$) of intestinal nematodes (65.5%) compared to dogs from 3- 6 months of age (36.8%) and dogs above 6 months of age (22.7%) (Table 4). Infection percent of *Toxocara canis* was highly significant ($P<0.01$) in puppies (15/29) compared to dogs from 3-6 month (3/19) and above 6 months (1/22) represented by 51.7%, 15.8% and 4.5% respectively. On the other hand, age was not statistically significant in infection rate of *Toxascaris leonina*, *Cystoisospora spp* and mixed infection.

According to season, samples were collected at two seasons, the infection percent showed no significant ($P> 0.05$) variation in cold season (21/41) in compared to hot season (10/29) that

represented 51.2% and 34.5%, respectively (Table 4).

The obtained data of the examined dogs of both sexes (38 male and 32 female) showed non statistically significant (P>0.05) variation in infection percentage between male (44.7%) and female dogs (43.8%) (Table 4).

According to the breeds, there was no significant (P>0.05) variation between local and exotic breeds. The infection percent of intestinal parasites in local and exotic breeds was 45.6% and 38.5% (27/70), respectively (Table 4).
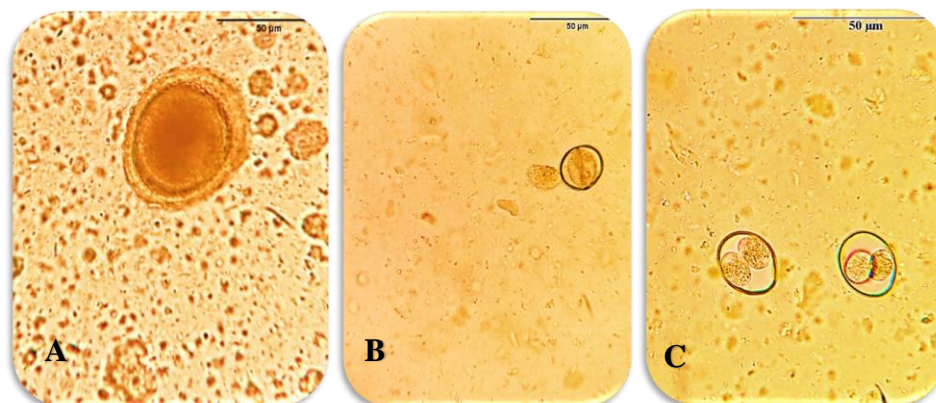

Fig. 1. Types of eggs in fecal examination: **A**) *toxocara canis* egg (400Xmagnification). **B**) *Toxascaris leonina* egg (400Xmagnification). **C**) *Isospora canis oocyst* (400Xmagnification).

Table 3: Overall infection percent of intestinal parasites in dogs.

Group	No. of animals (n= 70)	Percentage (%)
1. **Healthy control group**	39	55.7 %
2. **Positive group**	31	44.3 %
• *Toxocara canis*	19	27.1 %
• *Toxascaris leonine*	5	7.1 %
• *Cystoisosospora spp*	4	5.7 %
• Mixed infection (*Toxocara canis* with *Isospora canis*)	3	4.3 %

Table 4: Factors associated with gastrointestinal infection in dogs.

Factors		No. of dogs Examined	positive	Percentage (%)	P. value
Sex	Male	38	17	44.7 %	0.9340
	Female	32	14	43.8 %	
Age	1-3 M	29	19	65.5 %	0.0072**
	3-6 M	19	7	36.8 %	
	> 6 M	22	5	22.7 %	
Breed	Local	43	22	51.2 %	0.2164
	Exotic	27	9	33.3 %	
Season	Hot	29	10	34.4 %	0.1649
	Cold	41	21	51.2 %	

**highly significance difference at P <0.01

Hematological results:

There was high significant reduction (P<0.01) in the levels of erythrocytic parameters (Hb, PCV and RBCs) and significant increase (P<0.05) in the WBCs value in the parasitized in compared to the non-parasitized group of animals.

DLC was statistically non-significant except for eosinophils which significantly increased (P<0.05) in parasitized compared to non-parasitized animals. Hematological parameters for parasitized and non-parasitized dog populations are presented in Table 5.

Table 5. Mean ± SD and P-value of hematological parameters of healthy and intestinal parasites-infected dogs.

Parameter	Healthy group (n= 39)	Infected group (n= 31)	P. value
RBCs (x10^6 / mm3)	5.49 ± 0.72	4.94 ± 0.61	0.0015**
HB (g/dl)	12.49 ± 0.82	11.80 ± 1.02	0.0010**
PCV (%)	35.56 ± 2.92	32.16 ± 3.93	0.0001**
MCV (FL)	65.22 ± 4.08	65.06 ± 3.71	0.8690
MCH (Pg.)	23.37 ± 4.94	24.03 ± 1.79	0.4913
MCHC (%)	35.57 ± 5.51	37 ± 2.77	0.1982
WBCs (x10^3/ mm3)	10 ± 1.75	11.18 ± 1.95	0.0423*
Lymphocytes (%)	26.28 ± 1.95	25.61 ± 1.98	0.1668
Neutrophils (%)	59.1 ± 3.25	59.77 ± 1.77	0.3112
Monocytes (%)	6.72 ± 0.85	6.61 ± 0.68	0.5943
Eosinophils (%)	5.72± 1.15	6.45 ± 1.36	0.0190*
Basophils (%)	0.44 ± 0.55	0.52 ± 0.67	0.5866

Serum biochemical results:

Serum total protein (TP) and albumin values of parasitized group were found to be significantly lower (P<0.01) than non-parasitized one.

Both serum ALT and AST values were significantly increased (P<0.05) in parasitized than non-parasitized group whereas urea and creatinine values were non-significantly different between both groups. Biochemical data were presented in Fig. 2.

Discussion

Toxocara canis is the most predominant parasite in the present study with 27.1% infestation percent. This finding agreed with several studies. Kimura et al., (2013) and Kumar et al., (2014) reported *toxocara canis* infection percent of 21%, 22% 30%, 25%, and 28.93%, respectively. On the other side, Ugbomoiko et al., (2008)

and Radwan et al., (2009) reported higher percent of *Toxocara canis* infection 41.7% of domestic dogs in Nigeria and 53.4% in Egypt, respectively. This variation could be related to differences in management systems, health care and degree of environmental contamination with infective stages (Kumar et al., 2014).

The infection percent of *Toxascaris leonina* in the present study was 7.1% of the examined dogs. This result coincided with Trasviña-Muñoz et al., (2017) who reported infection percent of 5.5% for *Toxascaris leonina.*

The occurrence of *Cystoisospora spp.* was (5.7%) in 70 examined dogs. This result was close to results reported by Ferreira et al., (2005). The authors found that 5.6% of dogs affected by *Cystoisospora spp*. This result was lower than those reported by Anene et al., (1996) (18.3%), Buehl et al., (2006) (8.7%) and Nisar et al., (2009) (18%).
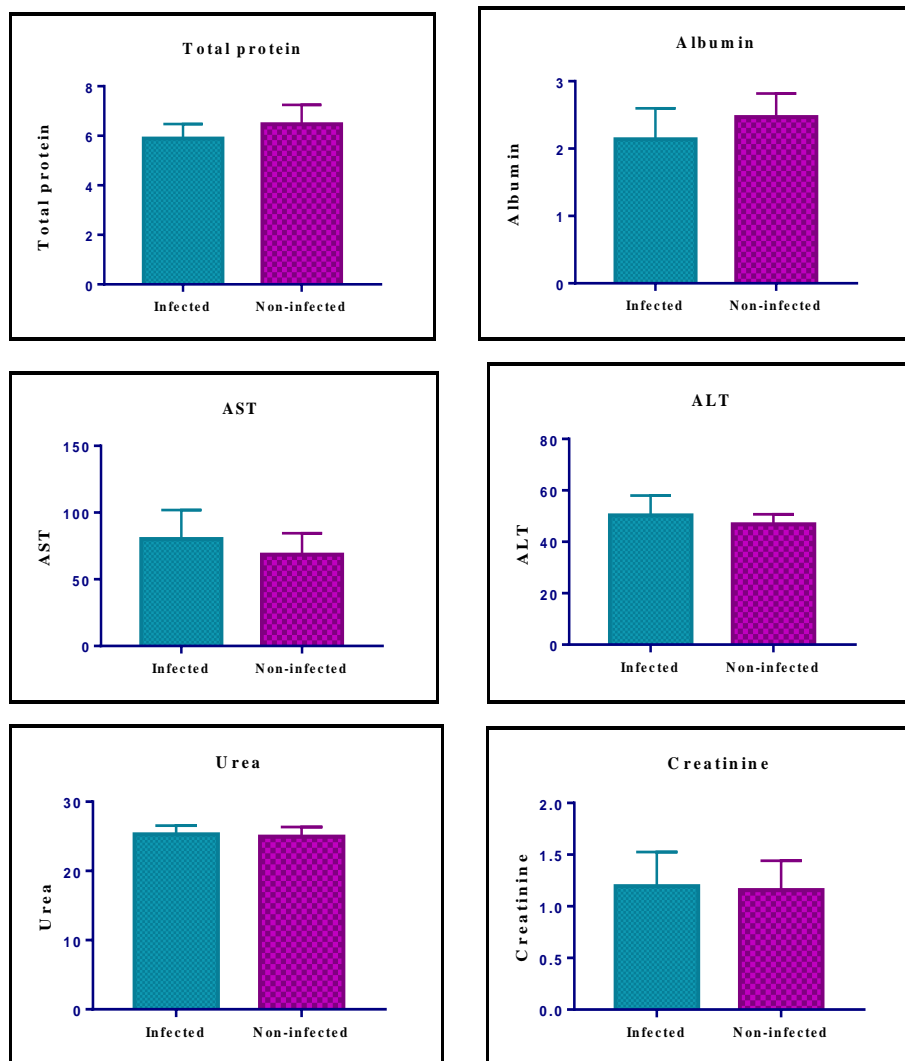
Fig. 2. Mean value (±SD) of serum total protein, albumin, AST, ALT, urea, and creatinine of healthy and infected dogs. * refers to significant difference at P <0.05, and ** refers to highly significant difference at P <0.01.

This could be attributed to the use of anti-coccidal drugs, geographic conditions, and awareness of the owners about the disease.

Age of animals played a very important role in infection percentage of gastrointestinal parasites in dogs. Puppies from 1-3 months showed significant (P<0.05) infection percent of intestinal parasites (65.5%) compared to (36.8%) in dogs from 3- 6 months and (22.7%) in dogs above 6 months. This result agreed with (Eslami et al., 2010 and Ahmed et al.,

2014). The authors attributed this finding to the age acquired specific immunity against parasites or to trans-placental or trans-mammary infection during the first few days of life (Epe, 2009). *Toxocara canis* and *Toxascaris leonina* were the predominant gastrointestinal nematodes among puppies. Similar findings have been reported by (Swai et al., 2010 and Kumar et al., 2014).

In the present study, there was no significant (P> 0.05) difference in infestation percent between male and

female dogs. Similar findings have been reported in previous studies (Awoke et al., 2011 and Abere et al., 2013). On the other side, Ahmed et al., (2014) and Davoust et al., (2008) indicated that female dogs were at risk of acquiring enteric parasitic infestation than male dogs. This may be due to the physiological peculiarities of the female dogs, which usually constitute stress factors thus reducing their immunity to infections (Swai et al., 2010)

This study showed no significant variation in infection percent between cold and hot season. This result agreed with (Oliveira-Sequeira et al., 2002).

Breeds were found to be a non-significant risk factor for occurrence of gastrointestinal parasitic infestation in dogs. (45.6%) in local breeds and (38.5%) in exotic breeds. Similar finding reported by (Swai et al., 2010 and Kumar et al., 2014).

Current study reveals that 68.6% (48/70) of examined dogs were apparently healthy, while 31.4% (22/70) of them showed signs of diseases. Out of 48 asymptomatic dogs, 9 animals (18.8%) were confirmed for enteric parasites infestation, while all symptomatic cases were infected with gastrointestinal parasites. This result confirming that absence of clinical symptoms is not an evidence for absence of infection. This finding agreed with (Stehr-Green et al., 1987 and Mohamed et al., 2009). Also, (Schmidt et al., 2016) reported that apparently healthy dogs at clinical examination were infected with nematodes suggesting that symptomatology-based diagnosis alone is inadequate.

Obtained hematological data revealed a significant reduction in the RBCs, Hb and PCV values in the infected group than in the healthy group. These results agreed with (Qadir et al., 2011; Kumar et al., 2014).

There was significant increase in WBCs in infected dogs indicating leukocytosis. This finding agreed with (Chattha et al., 2009 and Sharma et al., 2010). This increase might be related to liberation of histamine and histamine like substances from tissues damaged by the parasite effect.

Significant increase in eosinophils was observed in infected group indicating eosinophilia. Similar results obtained by (Ogunkoya et al., 2006 and Sharma et al., 2010). This increase could be attributed to the larval migration and defense mechanism against enteric parasites (Kirkova et al., 2005).

Generated biochemical data revealed a significant (P<0.05) decrease in total serum protein (TP) especially albumin level in infected group compared to healthy group. Similar trend has been reported earlier by (Kaymaz et al., 1999 and Nwoha et al., 2013). Hypoproteinemia is attributed to increase the serum leakage through the injured gut and to the interference with efficacy of absorption of the damaged intestine (Kumar et al., 2014).

In current study, both ALT and AST values were significantly increased in the infected group in compared to healthy group. This result agreed with (Hayden and Kruiningen, 1975 and Nwoha et al., 2013). This increase may be correlated to damage of liver by migrating larvae leading to increase hepatic permeability of those enzymes to blood stream (Kumar et al., 2014).

In conclusion, the overall percentage of intestinal parasites infestation in this study was 44.3% and *Toxocara canis* was the most common parasite with infection percent (27.1%) and it was found to be influenced by age. intestinal parasites, especially *toxocara canis*, were found to exert significant effects on hematological and biochemical parameters suggesting their importance as a health problem in dogs.

Conflict of interest statement

The authors declare that there is no potential conflict of interest.

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