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Research Article

PATHOLOGICAL AND PHYSIOLOGICAL ALTERATIONS INDUCED BY INTESTINAL HELMINTHS INFECTING DOMESTIC PIGEON, *COLUMBA LIVIA DOMESTICA*

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ABSTRACT

Four helminths were recorded in intestine of *Columba livia domestica*, pigeon; one nematode (*Ascaridia columbae*) and three cestodes (*Cotugnia polyacantha, Raillietina beveridgei and Raillietina echinobothrida*). 120 *C. L. domestica* pigeons were purchased from Ismailia, Egypt during the period from December, 2015 to November, 2016 and examined for presence of helminths, with highest prevalence of infection in summer (73%) and the lowest in winter (43.3%). The total prevalence of infection with helminths in males (64.8%) was higher than females (48.9%). Pigeons with greater weight revealed higher percentage of infection (61.1%). The intestine of the infected pigeons showed a number of histopathological alterations; coagulative necrosis of the villi columnar epithelial cells and infiltration of inflammatory lymphocytic cells as well as blood vessels congestion, leading to an increase in the concentration of MDA (Malondialdehyde as a marker for lipid peroxidation) in both liver and intestine tissues of the infected pigeons compared with uninfected ones. There was a significant decrease in the level of antioxidants (GSH, GST, CAT, and SOD) in the intestine ($P<0.05$), while the decrease was non- significant in liver of the infected pigeons. In conclusion, helminths infections affect physiological status of *C. L. domestica* and induced remarkable pathological changes which may result in economical loss for the breeders. Further attention should be considered regarding intestinal helminths infecting *C. L. domestica*. In addition, biological factors that play an essential role in affecting helminths community should be studied.

Keywords: Biological factors, *C. L. domestica*, Helminths, Histopathology, Oxidative stress.

INTRODUCTION

Helminths can parasitize a wide variety of birds (Tietz marques *et al.*, 2007). The domestic pigeon *Columba livia domestica* is considered one of the birds that can parasitized a by wide variety of Helminths (Marques, 2007). Moreover, helminths infection exhibits serious effects on the host tissue (Hoste, 2001). These parasites can induce definite histopathological effects such as; ulceration and sloughing of intestine mucosal epithelium, degeneration of villi, destruction of secretary glands of epithelium, infiltration of inflammatory cells and villi atrophy (Abed *et al.*, 2014).

Recently, a lot of pigeon deaths have been occurred due to presence of parasitic infections (Tanveer *et al.*, 2011). Gastrointestinal tract of infected pigeons always showed epithelium complications and immune disturbance of host leading to death (Basit *et al.*, 2006). The intestine of domestic pigeon can be influenced by a various histopathological changes as a result of parasitic infection as necrosis in the villi, glands and the muscularis mucosae layer, mononuclear and polymorph nuclear cells in the necrotized areas (Adang *et al*., 2010).

Oxidative stress is an imbalance between reactive oxygen species production and antioxidant defenses, that may cause tissue injury (Betteridge, 2000). Oxidative stress initiate a variety of auto-oxidative chain reactions on DNA, unsaturated fatty acids and proteins of cell membrane producing mutation, destruction of organelles and macromolecule (Sree & Padmaja, 2008).

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Oxidative stress can be assessed by measurements of reaction products of oxidative damage like lipid peroxidation and DNA oxidation that considered biomarkers for oxidative process that can be used to assess the degree of the damage and degradation of biomolecules and endothelial cell membranes (Betteridge, 2000; Torres ramos *et al*., 2009). These harmful effects can be reduced through antioxidant defences, which include an arsenal of endogenous and exogenous compounds that impede oxidant chain reactions by direct oxidants neutralization. Wang *et al.* (2008), Rizvi *et al.* (2012), and Sureshkumar *et al.* (2013) reported a decrease in the antioxidant activities in case of parasitic infection. So, our study aimed to investigate the biological factors possibly affecting prevalence of infection, pathological impact on the infected pigeons and the oxidative stress caused by helminths.

MATERIALS AND METHODS

Sample collection

120 domestic pigeon *(C. L. domestica)* were purchased seasonally (30 per each season) from a bird's market in Ismailia, Egypt during the period from December, 2015 to November, 2016.They were immediately transported to the laboratory and were killed by anesthesia using chloroform. Pigeons were dissected according to the method described by Al-Hussaini and Demian (1982). Sex (71 male and 49 female) and weights of the pigeons were determined. They were divided into two weight classes as follows; class 1 (100-250gm) (84) and class 2 (260-400gm) (36).Collected parasites from infected pigeons sorted into nematodes and cestodes. Identification of helminths was carried out according to Soulsby (1982). All experimental procedures involving animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (http://www.nap.edu/catalog/12910.html) and approved by the Research Ethics Committee (Section of Experimental Animals 1/2017) of the Faculty of Science, Suez Canal University, Ismailia, Egypt.

Histopathological studies

Small pieces of intestine of both infected and non-infected pigeons were fixed in Bouin's solution for 48 hr; post fixed (washed) in several changes of 70% alcohol, then dehydrated in ascending series of alcohol (70%, 80%, 90%, 100% and 100%); cleared in two changes of xylene and infiltrated with paraffin wax, then paraffin blocks of specimens were made. Histological sections were stained by Harris' haematoxylin and counterstained.

Oxidative stress evaluation

Prior to dissection, perfusion of intestine and liver tissues were carried out using a phosphate buffered saline solution (PBS), pH 7.4 containing 0.16 mg/ml heparin, homogenized in 5-10 ml cold buffer (100 mm potassium phosphate, pH 7.0, containing 2 mm EDTA) per gram tissue, centrifuged at 4,000 rpm for 15 minutes at 4 °C and collect supernatant. 0.5 ml of ice-cold extraction reagent was added to 1 ml of supernatant in glass test tube and vortex for at least 30 seconds, centrifuged at 4000 rpm at 20 °C for 10 minutes.

The supernatant homogenates were used calorimetrically for the determination of non-enzymatic antioxidants activities; reduced glutathione (GSH) (Beutler, 1963) and perioxidation expressed by malondialdhyde (MDA) (Kei, 1978). In addition, enzymatic antioxidants activities; superoxide dismutase (SOD) (Nishikimi *et al*., 1972), catalase (CAT) (Aebi, 1984), and glutathione s-transferase (GST) (Habig *et al.,* 1974) have been evaluated.

Statistical analysis

The prevalence of cestodes and nematodes infection in pigeons was recorded according to (Margolis *et al.,* 1982). Unpaired t- test was used for studying the significant differences between groups. All data were analyzed with the software packages Microsoft SPSS version 20, for statistical evaluation. Values of $P < 0.05$, $P < 0.01$ and $P < 0.001$ reflected levels of significance.

RESULTS

Season, host sex and host weight effects on prevalence of helminths

Prevalence of infection for both nematodes and cestodes in this study recorded the highest value in summer (73.3%) and the lowest in winter (43.3%). In winter, *A. columbae* revealed higher prevalence of infection (16.3%) compared to other helminths, while *C. polyacantha* revealed the highest prevalence of infection in both spring and summer (26.7%, 33.3%), respectively. *R. echinobothrida* recorded the highest prevalence of infection in autumn (26.7%) **(**Figure 1).

The total prevalence of infection with helminths in males (64.8%) was higher than females (48.9%). Prevalence of infection of *C. polyacantha* was the highest in both males and females (21.1%, 18.4%) respectively, while *A. columbae* revealed the lowest prevalence of infection in both males and females (11.3%, 8.2%), respectively (Figure 2). The total prevalence of infection with helminths in weight class 2 (260-400 gm) was (61.1%) which was higher than weight class 1 (100-250 gm) (55.6%). *C. polyacantha* revealed the highest prevalence of infection in weight class 1 (23.8%), *R. beveridgei* showed the highest prevalence of infection in weight class 2 (19.4%), while, lowest prevalence of infection in both weight class 1 and class 2 was for *A. columbae* (7.1%, 11.1%), respectively (Figure 3).

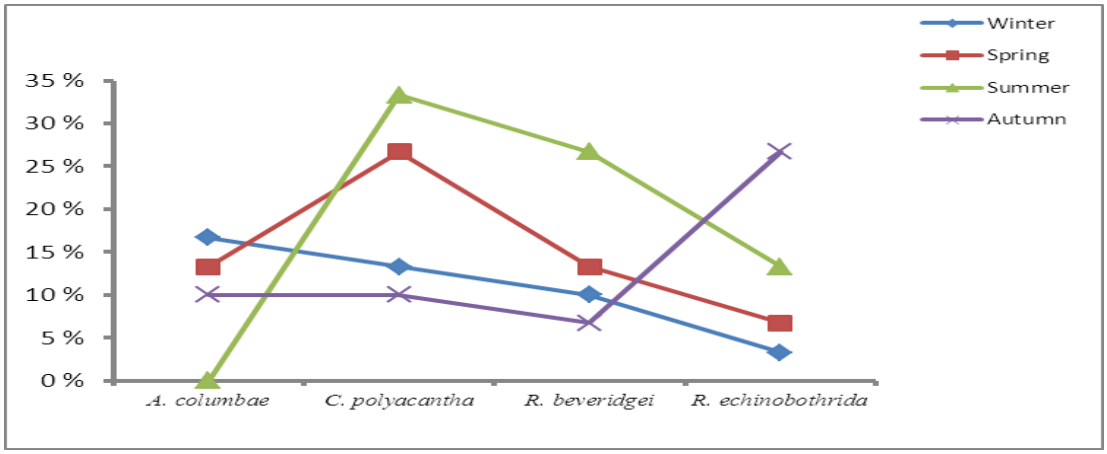

Figure 1. Seasonal dynamics of prevalence (%) of infection concerning helminths species infecting *C. L. domestica.*

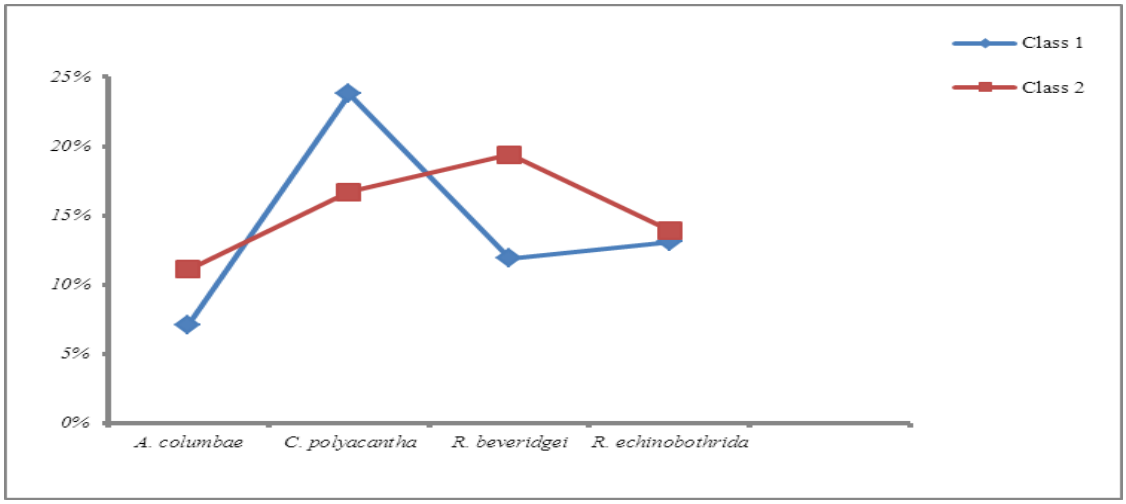

Figure 2. Sexual dynamics of prevalence (%) of infection concerning helminths species infecting *C. L. domestica.*

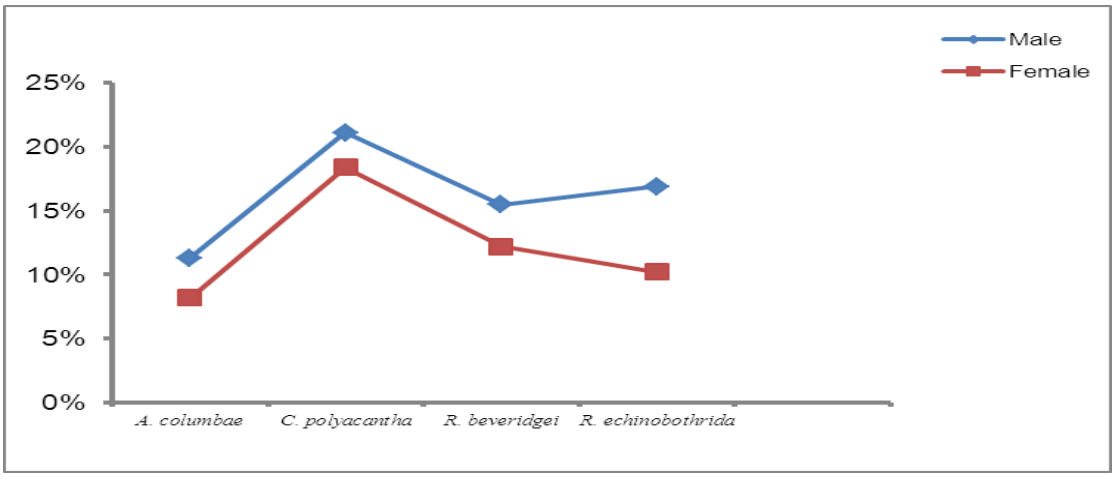

Figure 3. The prevalence (%) of infection with helminths species infecting *C. L. domestica* according to body weight.

Histopathological changes of the infected pigeons

The intestine of the domestic pigeon has three histological layers. They are from outside to inside; the serosa, the muscularis and the mucosa. Infected pigeon's intestine showed several histopathological changes which were: coagulative necrosis of the villi columnar epithelial cells**.** Different types of cestodes were seen in the lumen of intestine. Other histopathological changes caused by helminths were infiltration of inflammatory lymphocytic cells as well as blood vessels congestion (Figure 4).

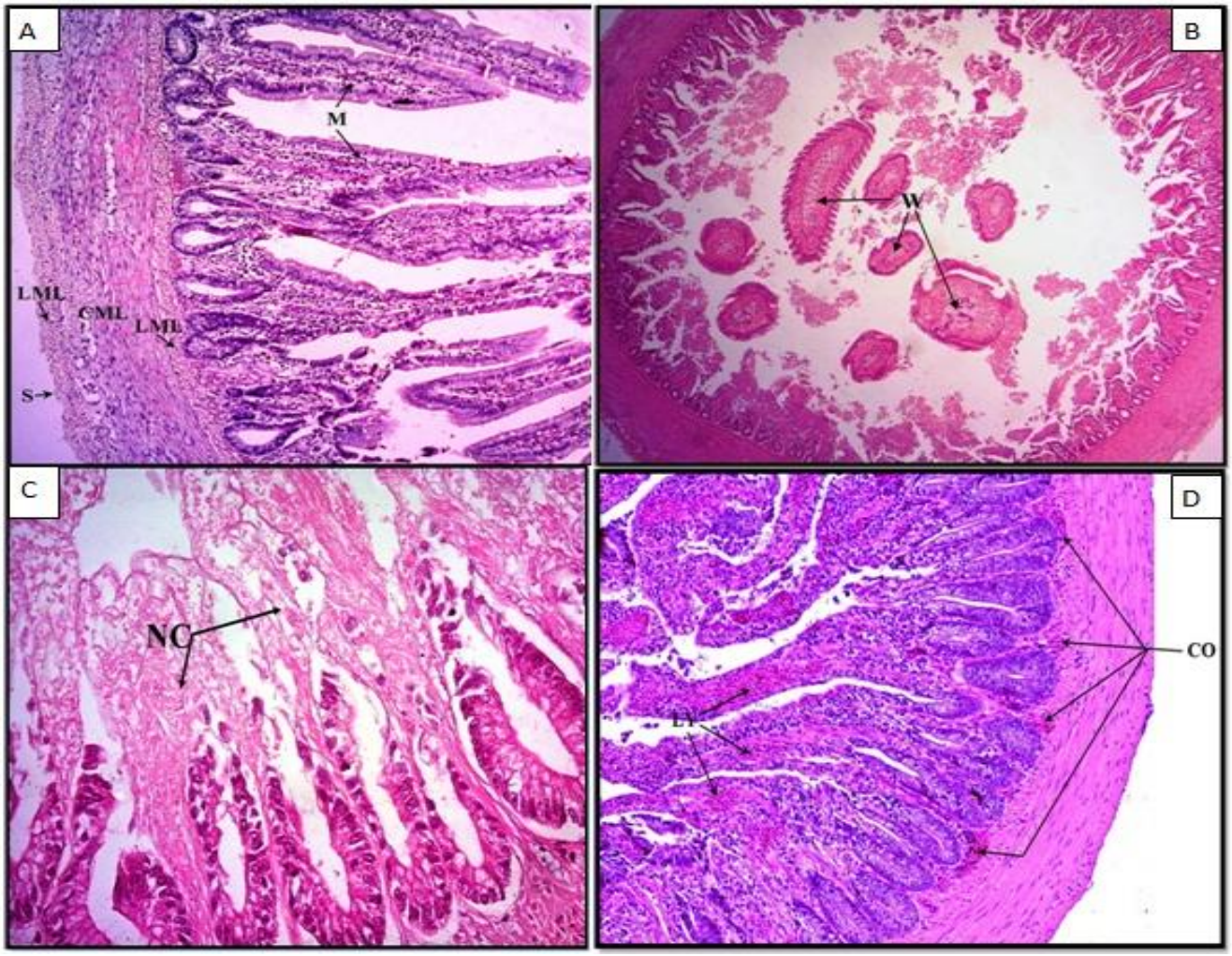

Figure 4. A. Photomicrograph showing cross section in the intestine of uninfected domestic pigeon revealing different intestine layers: mucosa (M), muscularis (LML: an outer longitudinal muscle layer, CML: a middle circular muscle layer and LML: an inner longitudinal muscle layer) and Serosa (S) (H&E) (X10). **B.** Cestodes parasites in the lumen of intestine (W) (H&E) (X5). **C.** Intestine showing coagulative necrosis (NC) of villi columnar epithelium (H&E), (X40). **D.** Intestine showing massive Infiltration of lymphocytic inflammatory cells (LY) and blood vessels congestion (CO) (H&E) (X10).

Oxidative stress and antioxidant status

MDA and antioxidants markers (GSH, GST, CAT, and SOD) were measured in the liver and intestine of the infected pigeons as well as the control ones. In liver, helminths caused significant increase in MDA levels (13.7±1.1) compared with uninfected pigeons (11.2±0.2).The level of GSH, and the activity of GST ($P<0.004$), CAT, and SOD ($p<0.005$) decreased in the infected pigeons (Table 1). It is worth nothing that the decrease of GST and SOD was statistically significant. Concerning intestine, also helminths caused significant increase in MDA levels in the intestine of the infected pigeons (18.6±2) compared with uninfected pigeons (9.7±1.5) ($P<0.05$). The level of GSH ($P<0.01$) and the activity of GST($P<0.01$), CAT, and SOD ($P<0.001$) decreased in the infected pigeons (Table 2).

Table 1. Liver antioxidant activities (mean ± S.E.) of pigeons infected with helminths compared to uninfected ones.

Groups	MDA mol/g	GSH mg/dl	GST U/g	CAT U/g	SOD U/mg
Infected	13.7±1.1	2.3±0.5	21.9±1.7*	0.8±0.03	33.5±1.3*
Uninfected	11.2±0.2	3.6±0.6	33±0.4	0.9±0.02	46.5±0.2

(*) Represents significant difference between infected group and uninfected group using student's unpaired t-test, * ($P < 0.05$).

Table 2. Intestine antioxidant activities (mean ± S.E.) of pigeons infected with helminths compared to uninfected ones

Groups	MDA mol/g	GSH mg/dl	GST U/g	CAT U/g	SOD U/mg
Infected	18.6±2.0*	3.6±0.6*	24.7±1.7*	0.6±0.0	32.7±0.06*
Uninfected	9.7±1.5	8±0.0	47.7±2.9	0.8±.03	92.7±3.8

(*) Represents significant difference between infected group and uninfected group using student's unpaired t-test, * ($P < 0.05$).

DISCUSSION

The prevalence of many infectious diseases fluctuates markedly through time, from short-term seasonal variations to complex population dynamics (Greenman *et al*., 2004)and (Altizer *et al*., 2006). The sensitivity of vectors to climatic conditions alter the dynamics of vector-borne diseases (Hess *et al*., 2002).Prevalence of infection for both nematodes and cestodes in this study recorded the highest value in summer (73.3%) and the lowest in winter (43.3%), this may attributed to the high abundance of cestodes intermediate hosts in the warm season and the birds become in contact with intermediate host such as beetles and house flies) in deep litters which are a source for food for birds (Anwar *et al*., 2000). Abed *et al.* (2014) reported high prevalence of infection with *Raillietina* sp. compared to other parasites in the infected domestic pigeons.

Investigation on sex-biased parasitism revealed that males tend to have significantly higher parasite prevalence and intensity than females within vertebrate hosts (McCurdy *et al.*,1998); and (Moore & Wilson, 2002). In this study, prevalence of infection revealed the highest in males. Male sex hormone testosterone is one of the probable causes of male–biased parasitism due to its immunosuppressive effects in some vertebrates, leading to increased susceptivity to parasite infections (Isomursu, Rätti, Helle, & Hollmén, 2006).

Impairing the absorbing power of intestine for nutrients and vitamins from the host is mainly due to intestinal endoparasites, producing nodule and severe enteritis (Anwar *et al*., 2000). Histopathological alterations in response to helminths infection was obvious in the present study. The intestine of the infected pigeons showed coagulative necrosis of the villi columnar epithelial cells and infiltration of inflammatory lymphocytic cells as well as blood vessels congestion. These findings were supported by Nayyef & Sabbar (2012)who noticed an infiltration of mononuclear inflammatory cells and increase in the number of goblet cells in the intestine of pigeon infected with *R.* echinobothrida. Also these results are in accordance with the results of and Ghobashy *et al.*(2016)who study the effect of helminths infection on the small intestine of *Coturnix coturnix.*

Lipid peroxidation considered as one of the main exhibitions of oxidative damage induced by the free radicals produced during various types of xenobiotic exposures or pathological conditions (Fu *et al*., 2013; Santamaría *et al*., 2002). Results showed an increase in the concentration of lipid peroxidation (MDA) in both liver and intestine tissues of the infected pigeons compared with uninfected ones. Perhaps due to free radicals which can initiate lipid peroxidation by damaging the cellular membranes of liver and intestine that emphasize the role of parasitism in producing oxidative stress. Among these, is the formation of (MDA) as a product of lipid peroxidation (Fu *et al.,* 2013). This confirmed by (Siwela *et al*., 2013) who stated that there was a higher level of MDA in the liver of ostrich infected with *Codiostomum struthionis* and other cestodes when compared to non-infected ones.

Decline of enzymatic and non-enzymatic antioxidants may be resulted from the rapid consumption and exhaustion of storage used in the neutralization of free radicals produced against parasites. GSH is considered one of the most essential antioxidants in the cell, representing a crucial role in cell protection regarding oxidative stress (Shan *et al.,* 1990). Results in the present study revealed that the infected pigeons showed decrease GSH level in liver and intestine. This decrease may relate to the loss of GSH that impair cellular antioxidant defences leading to

accumulation of reactive oxygen species (Kolodziejczykka *et al.*, 2005).

Catalyzing the conjugation of glutathione to electrophilic substrates is mainly the role GST, producing less reactive and more soluble compounds (Hubatsch *et al*., 1998). GST plays a physiological role in initiating the detoxification of potential alkylating agents (Ilavarasan *et al*., 2003). Our results revealed a significant decrease of GST in the liver and intestine of infected pigeons. This expected to be due to stress that plays a potential role in aggravating tissue diseases in tissue inflammation through the generation of ROS (Zaidi *et al*., 2005).

The activity of catalase (CAT) recorded in this study showed decrease in the liver and intestine of infected pigeons. Parasite metabolic products generate ROS species in infected animals making depletion in the antioxidant molecules fighting them. The decrease in the activity of the antioxidant superoxide dismutase (SOD) in the liver and intestine of infected pigeons may be to the fact that oxidative stress results in up regulation of the production of superoxide and H_2O_2. Also, various antioxidant enzymes are expressed depending on harmful ROS generated by the presence of parasite and various cytokines leading to a drop in the effectiveness of intestinal and hepatic protection against excessive levels of hydrogen peroxide (Rahman *et al.*, 2006).Wang et al. (2008)showed that plasma SOD activities decreased in the birds infected with *Eimeria tenell* than the uninfected group.

It has been shown that animal body had an effective mechanism to prevent the free radical induced tissue cell damage, this accomplished by a set of endogenous antioxidant enzymes and protein such as GST, SOD, CAT, GPX, GRD and GSH. Oxidative stress resulted from the imbalance between ROS production and antioxidant defense, leading to various pathological alterations (Uzun *et al*., 2007).

CONCLUSION

Helminths infections affect *C. L. domestica* on several levels; histopathological level as well as physiological level. In turn, it affects the health of birds (*C. L. domestica*) which may results in economical loss for the breeders. Further attention should be considered regarding intestinal helminths infecting *C. L. domestica.* Biological factors play an essential role in affecting helminths community considering prevalence of infection.

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