

PROSPECTIVE STUDY OF CLINICAL AND EPIDEMIOLOGICAL TRENDS OF INTESTINAL NEMATODES INFECTION IN DOGS IN UPPER EGYPT

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ABSTRACT

The study was carried out to determine the clinical course and epidemiological pattern of intestinal nematodes in dogs in Upper Egypt, and their impact on various hematological and biochemical parameters. A total number of 120 dogs (Age =1 month to 3 year), both sex and of different breed's size (small and large) were included in the present study. Parasitological, clinical, hemato-biochemical and epidemiological examinations were conducted. Microscopic analysis of fecal samples (n=120) revealed three types of intestinal nematodes: *Toxocara canis* (n=28), *Toxascaris leonina* (n =5), *Ancylostoma caninum* (n = 4) and mixed infection of *Toxocara canis* and *Dipylidium caninum* (n=2) with an overall infection rate 32.5% (39/120). *Toxocara canis* was predominate type of nematodal parasite infection in the studied population (23.3%). Puppies (0-<3 months) were greatly affected with intestinal nematodes (54.8%), in particularly *Toxocara canis* (45.2%). Infected puppies showed off food, pale mucous membrane diarrhea abdominal bloating with potbellied appearance and vomiting. Other group of examined animals (n= 54) showed no clinical signs (asymptomatic) however a portion of this group (25.9%) was infected suggesting that absence of clinical symptoms was not an evidence of the absence of infection. Sex and breed showed non-significant differences (P>0.05) on infection rate of intestinal nematodes but season had high impact on nematodal infection The infection rate showed significant (P<0.05) increasing in winter (42.3%) and autumn (43.8%) with a maximum infection rate reported in December (60%) and January (56.5%). Hematological data generated from 35 blood samples of infected and 40 samples of healthy dog populations showed significant decrease (P<0.05) in erythrocytic parameters (RBCs, Hb, PCV) and platelets count in infected group, whereas group differences for TWBCs, neutrophils, monocytes, lymphocytes, eosinophils and basophils were non-significant high. Biochemical data generated revealed significant decrease (P<0.01) in the value of serum total protein in infected group. Liver enzymes showed significant increase (P<0.01) in serum AST, ALT and ALP in infected group compared with healthy one. In conclusion, intestinal nematode in dogs was common in Upper Egypt with infection rate of 32.5% and *Toxocara canis* was the most common nematode infection (23.3%). They have significant effects on hematological and biochemical parameters suggesting their importance as a health problem in dogs.

Key word: Nematodes, *Toxocara canis*, *Toxocara leonina*, infection, dogs.

INTRODUCTION

In many parts of the world, the intestinal parasites of dogs receive considerable attention because dogs serve as host, reservoirs, carriers and transmitters of several pathogens (Traub *et al.*, 2005). Among of these parasites are *Toxocara canis*, *Toxascaris leonina*, *Ancylostoma caninum* and *Trichuris vulpis* (Ahmed *et al.*, 2014).

They are considered potential zoonotic and constitute a significant public health concern.

In Egypt, the risk of zoonotic infection related to domestic dogs is high as nowadays dogs are living with humans (Awadallah and Salem, 2015), with lack of public education about the risk of zoonotic diseases transmitted from dogs such as visceral and ocular larval migrans caused by *Toxocara canis* and cutaneous larval migrans caused by *Ancylostoma spp* (Awoke *et al.*, 2011).

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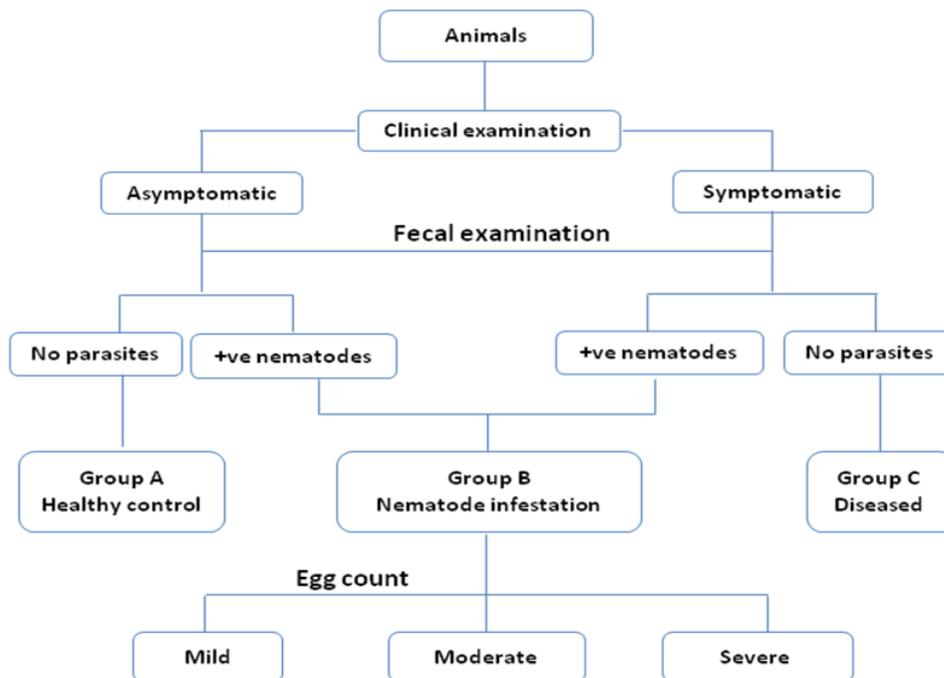


Figure 1. Study design: The examined dogs were classified into 3 groups (A, B, C). Group (A) healthy control dogs, group (B) dogs infected with intestinal nematodes and group (C) diseased but not infected with nematode dogs

In a recent study in Egypt reported 40% prevalence rate of enteric parasites infection in domestic dogs (Awadallah and Salem 2015). The high prevalence of enteric parasites infection in domestic dogs is due to absence of awareness about regular deworming of dogs (Ahmed *et al.*, 2014).

The clinical signs of intestinal nematodes in dogs are varied and occasionally some infected animals may present no symptoms (Awoke *et al.*, 2011). Intestinal nematodes in dogs may causes emaciation, anemia, constipation, intermittent diarrhea, a pot-bellied appearance and even death due to acute intestinal obstruction (Kumar *et al.*, 2014).

Toxocara canis is one of the most common intestinal parasites and worldwide distributed in dogs and cats. Adult worm may cause direct mechanical damage to the small intestine. Additionally, in older animals, larvae may migrate into other organs like lung, liver and muscles causing extra-intestinal lesion (Ballweber, 2001). There are different ways of transmission of toxocarasis including ingestion, transplacental and transmammmary depending upon the age and management of dogs (Kumar *et al.*, 2014).

Symptomatology based diagnosis alone is inadequate or impossible to diagnosis of intestinal nematodes (Carrol and Grove, 1986). Fecal examination and hemato-biochemical analysis is needed to make an accurate diagnosis.

Therefore, the present study aimed to determine the infection rate of intestinal nematodes in domestic dogs under field condition and their impact on the animal's health through assessing different clinical,

hematological and biochemical parameters in infected dogs.

MATERIALS AND METHODS

Animals: A total of 120 housed dogs (Age = 1 month to 3 year) from private clinic "Vet Clinic Sohag" at Sohag governorate, and "Animal Care Hospital" at Luxor governorate, southern Egypt, were examined for intestinal nematodes during the period from May 2016 to April 2017. Dogs were of both sexes and of different breeds (small and large sized dogs).

Animals were treated in accordance with guidelines established by the Faculty of Veterinary Medicine, Assiut University Committee on Animal Care. The objectives of the study were discussed with the owner and consent was taken before taking samples.

Study Design: Chart for the study design was presented in Figure 1.

Samples

Fecal samples: Fecal specimens were collected directly from rectum of examined dogs in clean, wide mouthed plastic containers with tight fitting lids and labeled with the dog's name, age, sex and date of collection. 10% formalin was used as preservative for the samples and transported to Parasitology Department, Faculty of Medicine, Assiut University for examination.

Blood samples: Blood samples were collected from cephalic vein according to Coles (1986) into two

small centrifuge tubes: one tube with anticoagulant (EDTA) for hematological studies and the other tube without anticoagulant for serum collection. Serum samples were stored at -20°C for estimation of serum biochemical parameters including serum total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).

Adopted Methods

Clinical examination: A clinical sheet was designed for examined animal contains owner data (name and phone number), animal's data (age, sex, breed and diet), case history, vaccination history, deworming history, the main complaint (Diarrhea, Distension, Abdominal pain, Nausea/vomiting and Itching in perianal region), general examination including abdominal examination and general appearance of illness (e.g. Pallor, emaciation and hair loss), physical examination (temperature, palpation and weight), consenting statement, suspected diagnosis and treatment.

Fecal examination: All fecal samples were examined macroscopically for color, blood, mucus, consistency and presence of adult nematodes or other endoparasites. Microscopic examination was performed using direct smear (Coles, 1986) and Formalin-Ether technique (Garcia and Bruckner 1988). Briefly, one drop of normal saline and one drop of iodine solution were placed on the same slide separately. Pin head part of the fecal material was then emulsified in saline and iodine, and each specimen was covered with a cover slip separately and examined. For confirmation of results, Formalin-Ether sedimentation technique was performed. Briefly, stool was mixed thoroughly with 10 ml formalin (10%) and left to stand for 30 minutes for adequate fixation. The formalin/stool mixture was strained through two layers of gauze and centrifuged for 10 minutes at 500g. Sediment was resuspended into physiological saline (0.85%) and centrifuged again for 10 min at 500xg. The supernatant was decanted and sediment resuspended in 10% formalin. Three ml of ethyl ether was added, the tube was shaken vigorously for 30 seconds and centrifuged for 10 min at 500xg. Four layers resulted: sediment; a layer of formalin; a plug of fecal debris and a layer of ether at the top. The plug of debris was freed by ringing with an applicator stick, formalin and ether fluid were decanted and a wet mount of remaining sediment was prepared for examination.

To obtain accurate information with regard to severity of infection, the fecal samples have been screened by Mc-Master technique (Coles, 1986) using known volume of stool suspension (2 x 0.15 ml). Briefly, 4g

of stool were suspended in 56 ml of saturated salt solution and passed through a sieve into a clean plastic container. While mixing vigorously, a sample of the mixture was taken with a pipette and transfer into both chambers of the McMaster slide. Then the total number of eggs under both chambers of the slide was counted and multiplied by 50 to obtain eggs per gram (EPG).

Maturation of *Toxocara canis* eggs: Suspension of suspected *Toxocara canis* eggs with 2.5 formalin/ringer was prepared and maintained at 25 °C at 98% humidity for 3 weeks for egg development to confirm *Toxocara canis* infection.

Blood examination: Whole blood samples were analyzed for complete blood picture (Hb, PCV, RBCs, TWBCs, and DLC) using CBC analyzer (Rayto 7600S). Serum samples were analyzed for TP, ALT, AST and ALP spectrophotometrically using commercial biochemical kits (Human Gesellschaft, Germany) and according to the recommendation of the expert panel of the International Federation of Clinical Chemistry (IFCC).

Statistical analysis: The data analysis was performed using of Excel spreadsheet (Microsoft Excel 2013) for descriptive statistics. Data were presented as Mean \pm SD. Significant of difference between health and diseased group was determined by T-test using the PRISM statistical software (Graph Pad Prism version 7.00, 2016).

RESULTS

Clinical signs: Out of 120 examined dogs, 54 dogs were apparently healthy showed no clinical sign (asymptomatic) and 66 dogs showed signs of disease (symptomatic). Symptomatic dogs were presented with one or more of the following signs: emaciation, potbellied appearance (Fig. 2), diarrhea, dull hair coat, hair shedding, off food, pale mucous membrane, abdominal bloating and vomiting. Fecal examination of both symptomatically and asymptotically dogs revealed 39 animals (group B) were infected with nematodes (25 symptomatic and 14 asymptomatic) (Table 1).

Fecal examination

Macroscopic examination

Macroscopic examination of the stool showed adult worm (white, 10–18 cm in length) of *Toxocara canis* (Fig. 3). In cases of mixed infection, macroscopic examination showed part of gravid segment of *Dipylidium caninum* (Fig. 4) with offensive odour.



Figure 2. Two-month-old puppy showed distended abdomen (potbellied appearance)



Figure 3. Adult worms of *Toxocara canis* (10–18 cm in length)



Figure 4. A gravid segment of *Dipylidium caninum* in a dog with mixed infection of *Dipylidium caninum* and *Toxocara canis*

Microscopic examination

Microscopic examination of the stool revealed presence of eggs of *Toxocara canis*, *Toxascaris leonina* and *Ancylostoma caninum*. Egg of *Toxocara canis* showed sub-spherical, yellow-brown outer shell that is roughly pitted, 75–90 μm , containing dark, single cell that fills interior of egg (Fig.5-a). Egg of *Toxascaris leonina* was sub spherical, light, and colorless with a smooth outer shell, 75–85 \times 60–75 μm in size, single cell present, which does not fill the egg (Fig.5-b). Egg of *Ancylostoma caninum* was thin-shelled, oval, 55–90 \times 34–45 μm , containing morula with 2–8 cells (Fig.5-c). There was a mixed infection contains eggs of *Toxocara canis* and *Dipylidium caninum*. Egg of *Dipylidium caninum* was approximately 50 μm in diameter, present in packets in (Fig. 5-d).

To confirm *Toxocara canis* egg in some cases of undetermined egg microscopically, stages of development of *Toxocara canis* was monitored in *in vitro* experiment. Samples were suspended and mixed

with 2.5% formalin and maintained at 25 °C at 98% humidity. Fully embryonated eggs of *Toxocara canis* were recorded after 3 weeks. During this period, developmental stages of *Toxocara canis* were observed (Fig. 6).

Infection rate and factors affecting the infection of intestinal nematodes

In the present study, an overall percentage of intestinal nematodes infection was found to be 32.5% (39/120). The infection rate was 33.75% (27/80) in Sohag and 30% (12/40) in Luxor governorate. Three types of intestinal nematodes were detected: the predominant one was *Toxocara canis* (23.3%) followed by *Toxascaris leonina* (4.2%) then *Ancylostoma caninum* (3.3%). Two of examined dogs (1.7%) had infected with more than one parasite (*Toxocara canis* and *Dipylidium caninum*) (Table 1&2, Fig. 7a). The positive samples were further classified according to total egg count into, high (28.2%), moderate (33.3%) and mild (38.5%).

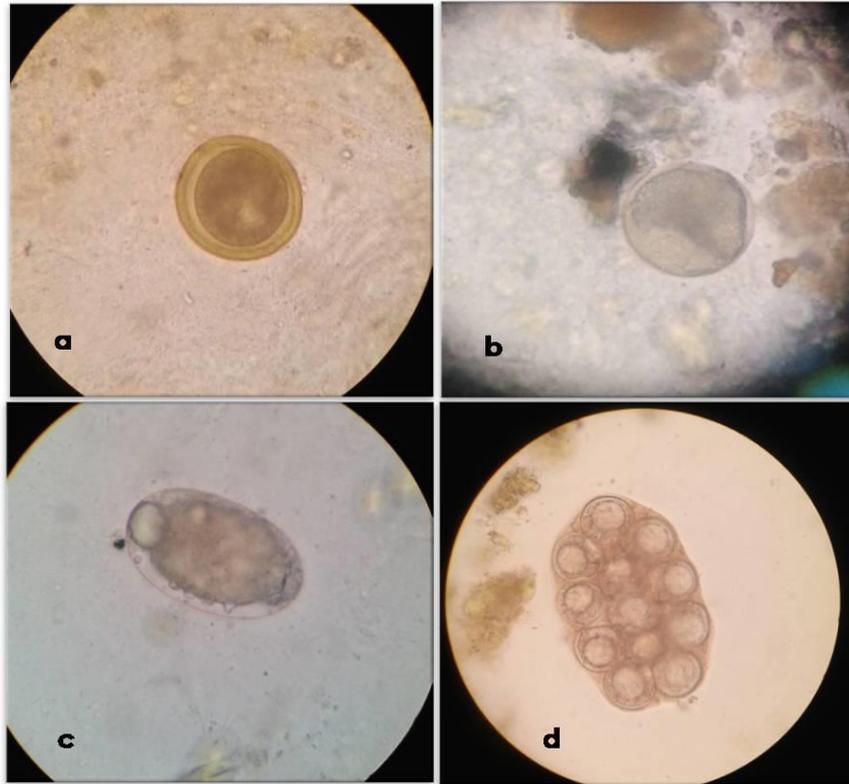


Figure 5. Types of eggs in fecal examination: a) *Toxocara canis* egg (x40) showed sub-spherical, yellow-brown outer roughly pitted shell 75–90 µm in size. b) *Toxascaris leonina* egg (x40) showed sub-spherical, light, and colorless with a smooth outer shell, 75–85 × 60–75 µm in size, single cell present. c) *Ancylostoma caninum* egg (x40) showed thin-shelled, oval, 55–90 × 34–45 µm in size, containing morula with 2–8 cells. d) Egg packet of *D. caninum* eggs (40x) containing at least 12 eggs

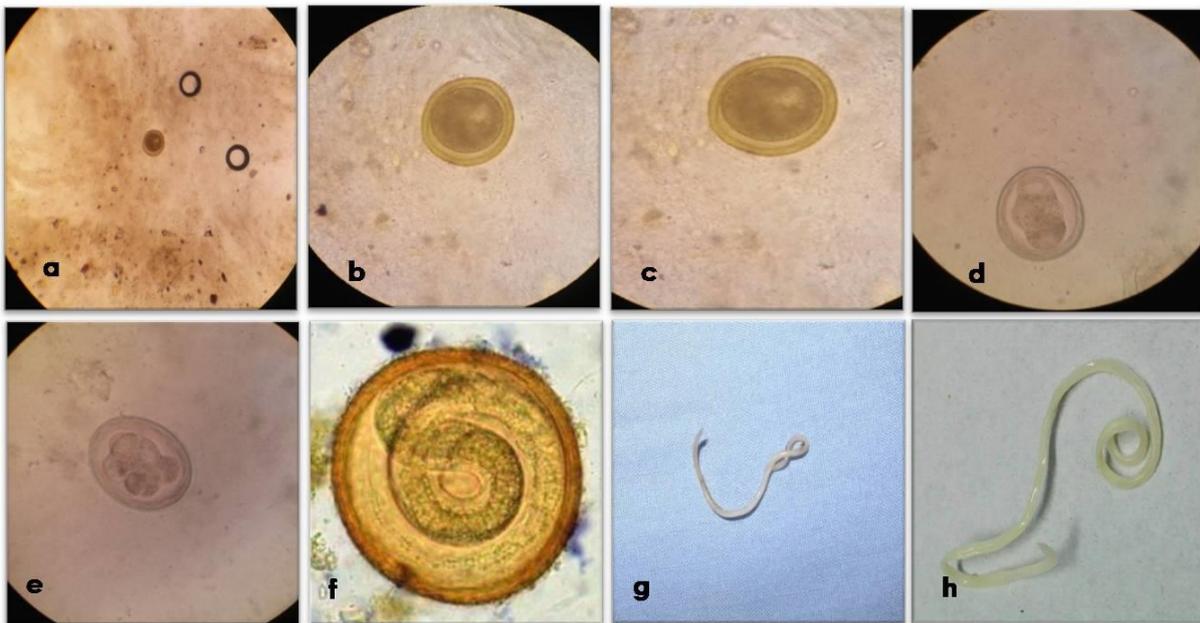


Figure 6. Stages of development of *Toxocara canis*. a) *Toxocara canis* (x10) egg with immature one cell stage embryo. b) *Toxocara canis* (x40) egg with immature one cell stage embryo. c) *Toxocara canis* egg (x100) with immature one cell stage embryo. d) *Toxocara canis* egg (x40) after 1 weeks of development. e) *Toxocara canis* egg (x40) after 2 weeks of development. f) *Toxocara canis* (x100) mature egg containing 2nd stage larvae after 3 weeks. g) *Toxocara canis* male (canine roundworm) from a puppy 4-10 cm long, the posterior end of the male is curved ventrally. h) *Toxocara canis* female (canine roundworm) from a puppy 5-18 cm long

Table 1. Overall infection rate of intestinal nematodes in dogs

| Group | No. of Animal (n= 120) | Percentage |
|--|------------------------|------------|
| 1. Negative group | 81 | 67.5 |
| • Healthy control group (A) | 40 | 33.3 |
| • Diseased non-nematodal infection group (C) | 41 | 34.2 |
| 2. Positive group (Nematodal infection, group B) | 39 | 32.5 |
| • <i>Toxocara canis</i> | 28 | 23.3 |
| • <i>Toxascaris leonina</i> | 5 | 4.2 |
| • <i>Ancylostoma caninum</i> | 4 | 3.3 |
| • Mixed infection (<i>Toxocaracanis with Dipylidium caninum</i>) | 2 | 1.7 |

Table 2. Infection rate of *nematodes* in symptomatic and asymptomatic examined dogs

| Parasite | Symptomatic (n = 66) | Asymptomatic (n = 54) |
|----------------------------|-------------------------|--------------------------|
| | Positive (n=25) | Positive (n=14) |
| <i>Toxocara canis</i> | 19 (28.8%) | 9 (16.7%) |
| <i>Toxascaris leonina</i> | 3 (4.6%) | 2 (3.7%) |
| <i>Ancylostoma caninum</i> | 1 (1.5%) | 3 (5.6%) |
| <i>Mixed Infection</i> | 2 (3%) | 0 |

The investigated factors affecting infection rate of intestinal nematodes were season, age, sex and breed.

According to season, samples were collected at the four seasons, the infection rate showed significant ($P < 0.05$) increasing in winter (11/26) and autumn (14/32) seasons that represented 42.3% and 43.8%, respectively and was less infection rate in summer (3/12) and spring (11/50) represented 25% and 22%, respectively. The highest infection rate was recorded in December (60%) and January (56.5%) while the least infection rate was recorded in July (16.7%) (Fig. 7b).

According to age, puppies from 1-3 months showed higher significant infection rate ($P < 0.001$) of intestinal nematodes (54.8%) compared to dogs from 3- 6 months (26.3%) and above 6 months (15%) (Fig. 7c). Infection rate of *Toxocara canis* was highly

significant ($P < 0.001$) in puppies (45.2%) compared to dogs from 3-6 month (15.8%) and above 6 months (7.5%). On the other hand, age was not statistically significant in infection rate of *Toxascaris leonina*, *Ancylostoma caninum* and mixed infection however *Toxascaris leonina* infection rate was higher (7.1%) in puppies less than 3 month. *Ancylostoma caninum* infection was higher in older dogs recorded 5.3% (Fig. 7c).

The examined dogs were of both sexes (80 male and 40 female). Obtained data showed no statistically significant ($P > 0.05$) difference in infection rate between male (30%) and female dogs (37.5%) (Fig. 7c). According to the breeds, there was no significant ($P > 0.05$) variation between large and small breeds. The infection rate of intestinal nematode in large and small breeds was 32.7% and 31.8%, respectively (Fig. 7c).

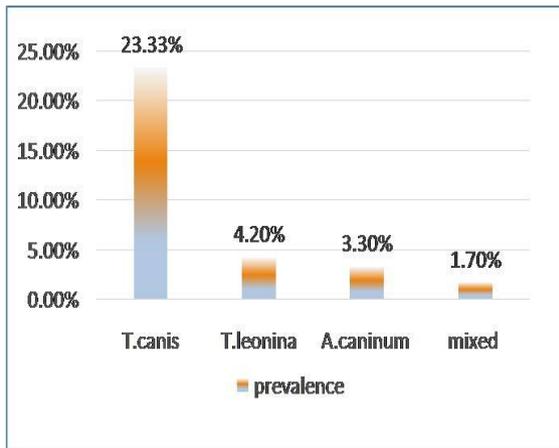


Figure 7a. Infection rate of intestinal nematodes in examined dogs

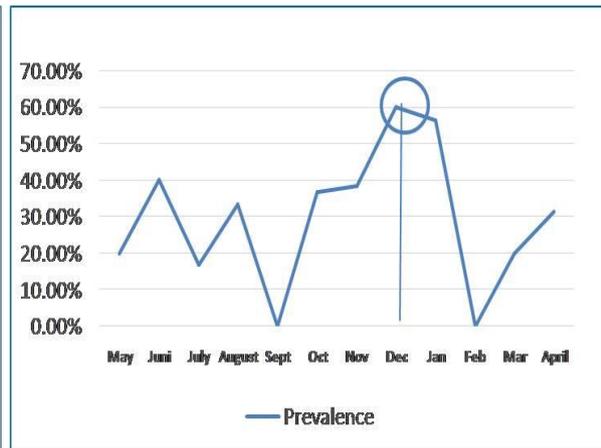


Figure 7.b. Prevalence of intestinal nematodes according to months

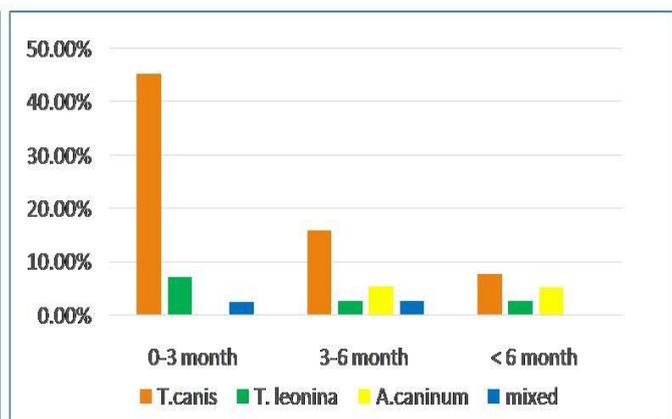
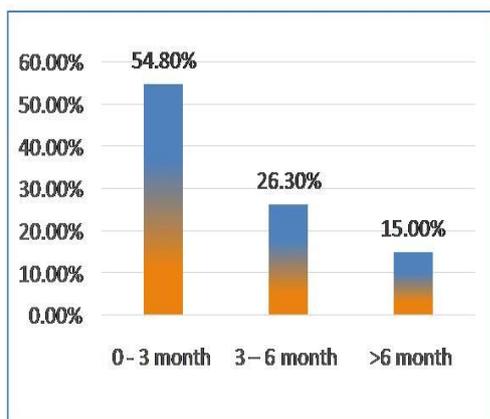
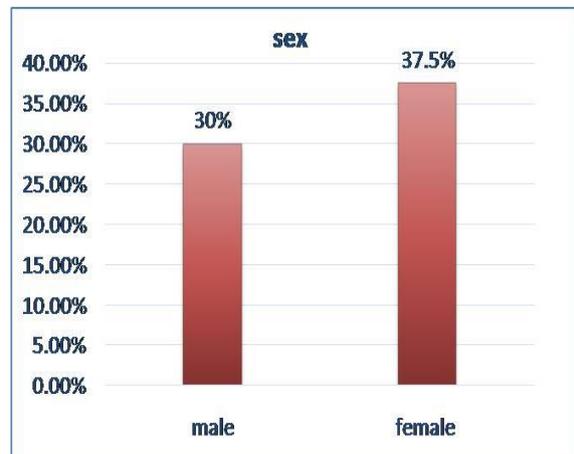
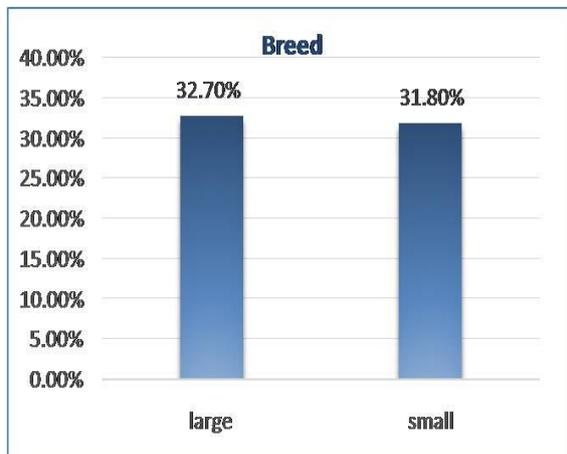


Figure 7.c. Infection rate of intestinal nematode according to breed, sex and age in examined dogs

Hematological and blood biochemical analysis

Hematological finding: Hematological data generated from 35 blood samples of the infected (group B) and of 40 samples of healthy one (group A) dog populations revealed significantly decrease ($P < 0.05$) in the levels of erythrocytic parameters (Hb,

PCV and RBCs) in infected group compared with healthy group whereas TWBCs and DLC were statistically non-significant increase. A significant increase ($P < 0.05$) in platelets was also observed in parasitized compared to non-parasitized animals. Data were presented in Table 3.

Table 3. Mean, SD and P-value of hematological parameters of healthy and intestinal nematodes infected dogs

| Parameter | Ref. Range | Healthy group (A) (n= 40) | Infected group (B) (n= 35) | P. value |
|---|-------------|------------------------------|-------------------------------|----------|
| RBCs (x10 ⁶ / mm ³) | 5.5 – 8.5 | 5.76±0.89 | 5.00 ± 0.60 | 0.0155 |
| HB (g/dl) | 12.0 -18.0 | 12.78±2.08 | 10.95 ± 1.60 | 0.0167 |
| PCV (%) | 37 – 55 | 38.29±5.78 | 32.95± 4.99 | 0.0484 |
| MCV (fL) | 60 – 77 | 67.10±5.07 | 65.87± 5.76 | 0.57753 |
| MCH (Pg.) | 19.5 - 24.5 | 22.37±1.57 | 21.91 ± 2.00 | 0.5948 |
| MCHC (%) | 32 – 36 | 33.46±1.65 | 33.22 ± 1.45 | 0.5556 |
| Platelets (x10 ³ / mm ³) | 200-900 | 234.43±83.75 | 306.31± 73.04 | 0.0259 |
| TWBCs (x10 ³ / mm ³) | 6 – 17 | 9.92±3.18 | 10.63 ± 4.20 | 0.6276 |
| Lymphocytes (%) | 12 – 30 | 30.71±7.87 | 29.38 ± 8.28 | 0.6922 |
| Neutrophils (%) | 60 – 70 | 61.29±9.97 | 61.69 ± 10.25 | 0.9318 |
| Monocytes (%) | 3 – 10 | 3.50±3.52 | 3.77 ± 3.06 | 0.8313 |
| Eosinophils (%) | 2 – 10 | 3.21±2.72 | 4.0± 3.92 | 0.4661 |
| Basophils (%) | 0 - 1 | 0.20±0.85 | 0.31± 0.48 | 0.3049 |

Significance: (P ≤ 0.05)

Serum biochemical findings

Biochemical data generated from 35 serum samples of infected group (B) and 40 samples of healthy group (A) dog populations were presented in Fig. 8. Serum total protein (TP) value of infected group (B) was found to be significantly lower (P<0.05) than healthy one (A). Both serum ALT and AST values were significantly increased (P<0.05) in infected group. Moreover, a highly significant increase (P<0.01) in ALP in infected group compared with healthy one was recorded.

DISCUSSION

Infection rate and risk factors

In the current study, an overall infection rate of intestinal nematodes in examined dogs was found to be 32.5% (39/120) in Sohag and Luxor governorate. A study conducted by Awadallah and Salem (2015) in Egypt revealed a similar level of infection (30%). Results obtained by Katagiri and Oliveira-Sequeira (2008); Coman *et al.* (2009); Panigrahi *et al.* (2014)

reported prevalence rate ranging from 8.7-36.6%. On contrary, lower and higher results were obtained across the world. The infection rate in Ethiopia (Awoke *et al.*, 2011) India (Sharma *et al.*, 2011) and Nepal (Satyal *et al.*, 2013) 14.7% and 19.5%, and 6.7%. Higher infection rate of enteric parasitic infection among the examined dogs was reported in South Africa 76% (Minnaar *et al.*, 2002), 50% in Hungary (Fok *et al.*, 2001), 85% in Mexico (Eguia-Aguilar *et al.*, 2005), 71% in Spain (Martinez-Moreno *et al.*, 2007), 68.4% in Nigeria (Ugbomoiko *et al.*, 2008) and 39.2% in Japan (Kimura *et al.*, 2013). The wide range of endoparasites infection rate may be related to geographical location, status of animal ownership, sampling protocols, demographic factors, anthelmintic usage, and diagnostic techniques (Katagiri and Oliveira-Sequeira, 2008). Also, the difference in the frequency of endoparasitic infection between places or countries is possibly due to the differences in climatic factors required for the biology of the parasites, veterinary

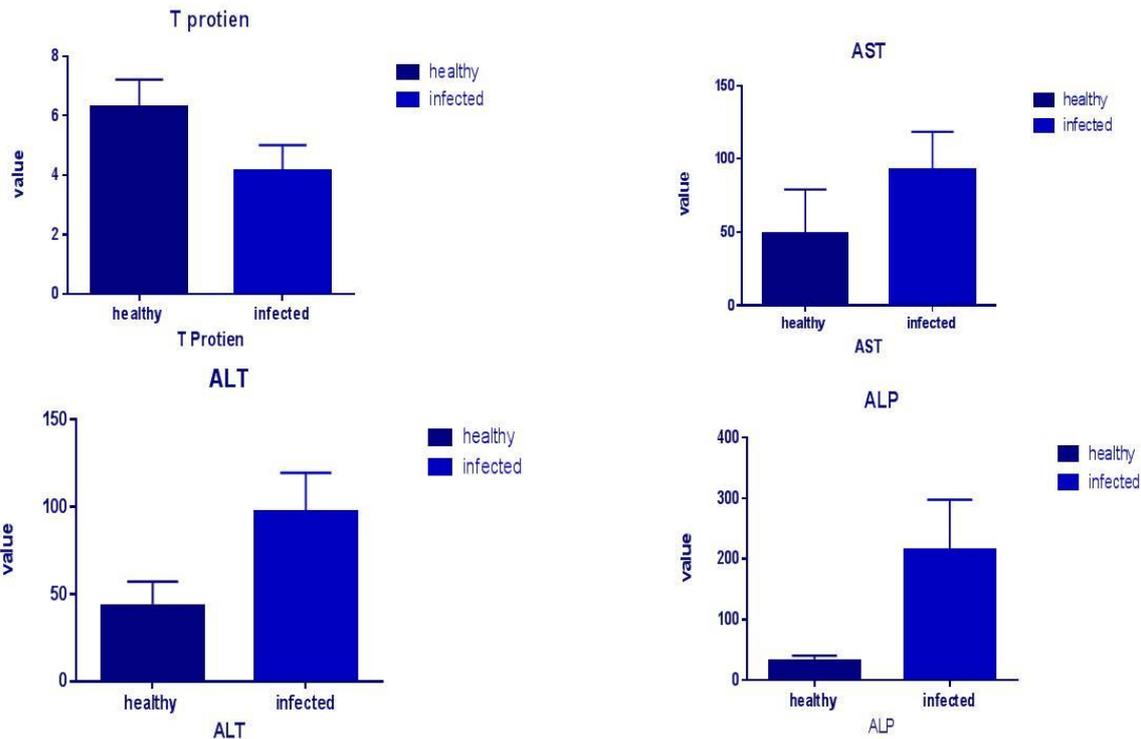


Figure 8. Mean value (\pm SD) of serum total protein, ALT, AST and ALP of healthy and infected dogs

facilities and public awareness to take care of the dogs (Abere *et al.*, 2013). The higher infection rate in this study could be attributed to less concern of regular deworming measure in Upper Egypt.

Toxocara canis is the most prevalent nematode parasite in the present study with 23% infection rate. This finding was in agreement with several studies. Studies conducted in Hungary, Germany, Italy, Ethiopia, Iran, Pakistan, Japan and India reported infection rate of 30.1%, 22.4%, 33.6%, 21%, 22%, 30%, 25%, and 28.93%, respectively (Fok *et al.*, 2001; Barutzki and Schaper 2003; Habluetzel *et al.*, 2003; Yakob *et al.*, 2007; Eslami *et al.*, 2010 and Chattha *et al.*, 2009 and Kimura *et al.*, 2013 and Kumar *et al.*, 2014). On contrary, Ugbomoiko *et al.* (2008) and Radwan *et al.* (2009) reported higher rate of adult *Toxocara canis* infection (41.7%) of domestic dogs in Nigeria and 53.4% in Egypt, respectively. Ahmed *et al.* (2014) reported lower infection rate of *Toxocara canis* in Alexandria, Egypt (0.8% of police dogs and 5% of house dogs). This variation could be attributed to differences in management systems, health care and degree of environmental contamination with infective stages and exposition to natural infection more than owned dogs. Studies reveal that dog's well cared for by their owners and given veterinary attention has lower incidence of intestinal helminths than dogs lacking such privilege (Kumar *et al.*, 2014).

In the present study, the infection rate of *Toxascaris leonina* was 4.2% of the examined dogs. The obtained results coincided with Awadallah and Salem (2015) in Qalyubia and Sharqia, Egypt. The authors

reported infection rate of 3.07% for *Toxascaris leonina* infection in dogs. Lower infection rate of *Toxascaris leonina* was reported by Barutzki and Schaper (2003), Schuster *et al.* (2009) and Lefkaditis *et al.* (2009) in Germany (1.8%), United Arab Emirates (0.8%) and Northern Greece (1.3%), respectively. On contrary, higher infection rate was reported by Dalimi *et al.* (2006) and Adinezadeh *et al.* (2013) as 32.53% and 53% of the examined stray dogs, respectively.

The occurrence of *Ancylostoma caninum* was (3.3%) in 120 examined dogs. This result was close to results reported by Ahmed *et al.* (2014). The authors found that 1.7% of police dogs affected by *Ancylostoma spp.* and 0% in housed dogs. In Japan, Kimura *et al.* (2013) reported 1.9% of examined dogs infected with *Ancylostoma spp.* However, the infection rate of *Ancylostoma caninum* in the present study was lower than those reported by Awadallah and Salem (2015) in Egypt (6.15%). Katagiri and Oliveira-Sequeira (2008) and Ugbomoiko *et al.* (2008) reported infection rate of *Ancylostoma spp.* of 37.8% and 16.9% in Brazil and Nigeria, respectively. This result may indicate that *Ancylostoma caninum* does not commonly infest dogs in Egypt. This may be due to the differences in climatic factors required for survival and perpetuation of the parasite.

Age of animals played a very important role in infection rate of intestinal nematodes. Among the different age groups studied (1-3, 3-6 and >6 month age), puppies from 1-3 months showed significant ($P < 0.05$) infection rate ($P < 0.001$) of intestinal

nematodes (54.8%) compared to (26.3%) in dogs from 3- 6 months and (15%) in dogs above 6 months. Higher infection rate of enteric parasitic infection in puppies than adults were previously reported (Eslami *et al.*, 2010, Abere *et al.*, 2013; and Ahmed *et al.*, 2014). The authors attributed this finding to the age acquired specific immunity against parasites or probably as a result of multiple re-infections. Moreover, related the highest occurrence in puppies to trans-placental or trans-mammary infection during the first few days of life (Katagiri and Oliveira-Sequeira, 2008). *Toxocara canis* and *Toxascaris leonina* were prevalent intestinal nematode among puppies. Similar findings have been reported in previous studies (Luty, 2001; Senlik *et al.*, 2006; Coman *et al.*, 2009; Swai *et al.*, 2010; Kumar *et al.*, 2014 and Panigrahi *et al.*, 2014). Puppies are usually born with or acquire *Ascarids* infection early in life through trans-mammary and trans-placental transmission (Ugochukwu and Ejimadu, 1985; Hendrix *et al.*, 1996, Swai *et al.*, 2010).

In the present study, there was no statistically significant ($P > 0.05$) difference in infection rate between male and female dogs. This finding was in an agreement with Awoke *et al.* (2011) and Mirzaei and Fooladi (2012) and Abere *et al.* (2013). On contrary, Umar (2009) and Ahmed *et al.* (2014) indicated that female dogs were more likely contracting intestinal nematodes 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). The variation between results possibly reflects the variation in host sex and age behavioral patterns or in food habits in dogs under studies (Radwan *et al.*, 2009). This means that both sexes are susceptible to have the infection with intestinal nematodes if they are under the same condition.

Additionally, the effect of breed and season on the infection rate of intestinal nematodes was investigated. The present study revealed no significant ($P > 0.05$) variation between large and small-breed dogs. Similar results were obtained by Swai *et al.* (2010); Ahmed *et al.* (2014) and Kumar *et al.* (2014). This indicates that all breeds have equal chance of acquiring the infection if they are exposed to infected materials. On other hand, the present study showed that season played an important role for nemoatodal infection. The infection rate showed significant ($P < 0.05$) increasing in winter 42.3% and autumn 43.8% with a maximum infection rate reported in December (60%) and January (56.5%). This low rate of infection in summers could possibly be due to the hot, arid climatic conditions which might render eggs non-infective. On contrary, Oliveira-Sequeira *et al.* (2002) did not find a relationship between the occurrence of gastro-intestinal nematodes and season.

Clinical signs

In the current study, 55% (66/120) of examined dogs showed sign of diseases while 45% (54/120) had no signs of diseases. Out of 66 symptomatic dogs, 25 animals (37.9%) were confirmed for nematodal infection while 14 cases out of 45 asymptomatic dogs (31%) showed no signs (asymptomatic) suggesting that absence of clinical symptoms is not an evidence of absence of infection. This is in agreement with Stehr-Green *et al.* (1987) and Mohamed *et al.* (2009) who reported that observable clinical signs were not predictive for the presence of parasites in the stool.

Symptomatic conformed cases of *Toxocara canis* showed potbellied appearance, stunted growth, intermittent diarrhea and constipation. These signs were evident in infected puppies suggesting that younger dogs are sensitive to infection than older one. Similar observations were reported by Mohamed *et al.* (2009) and Kumar *et al.* (2014). Schmidt *et al.* (2016) reported that the examined dogs infected with nematodes were apparently healthy at clinical examination and not showing clinical signs suggesting that symptomatology-based diagnosis alone is inadequate.

Macroscopic and microscopic examination of fecal samples

Macroscopic examination of stool was performed to detect odor, color, blood, mucus, consistency and adult or segment of worm. In the current study most cases of nematode infection had offensive odor and some had blood cells. Adult worm of *Toxocara canis* (looks like small snakes of 10–18 cm in length) and gravid segment of *Dipylidium caninum* (looks like a grain of rice) were detected macroscopically in the present study pinpoint the importance of gross examination of stool in such cases. Microscopic examination of stool had the ability to detect three types of nematodes: *Toxocara canis*, *Toxascaris leonina* and *Ancylostoma caninum*. To obtain information with regard to degree of infection, the fecal samples have been screened using Mc-Master technique for total egg count; however it did not give an accurate picture about the severity of disease. Maximov (2002) reported that egg counts are less valuable in making judgment about the clinical condition of the individual animal, since many factors affect the accuracy of fecal egg counts. The consistency of feces, the, type of the parasite, and immunity status of the host are affecting the total egg count. In vitro maturation experiment was conducted in this study to confirm *Toxocara canis* infection. Fully embryonated eggs for *Toxocara canis* was reported after 3 weeks.

Hematological and biochemical analysis

Hematological data generated from 35 blood samples of the infected group (B) and of 40 samples of the healthy group (A) dog populations revealed that the levels of erythrocytic parameters (Hb, PCV and RBCs) were significantly lower ($P < 0.05$) in the

infected group than in the healthy group. There was non-significant increase in TWBCs, eosinophils, neutrophils, monocytes, and basophiles, and a decrease in lymphocytes. Group differences for TWBCs and DLC were statistically non-significant. A significant increase ($P < 0.05$) in platelets were also observed in the parasitized compared to the non-parasitized animals. Similar results obtained by Sharma *et al.* (2011) and Panigrahi *et al.* (2016). This increase in TWBCs might be due to liberation of histamine and other histamine like substances from the damaged tissues. These values were also in close agreement with the findings of Karadam *et al.* (2008) and Lai and Chen (2007). The slight increase in eosinophils could be attributed to the larval migration and defense mechanism against endoparasites, (Kirkova *et al.*, 2005). Similar observation was also reported by Leder and Weller (2000).

There was also a significant decrease ($P < 0.05$) in PCV, HB and RBCs. Similar finding were obtained by Chattha *et al.* (2009) Kumar *et al.* (2014).

Biochemical data generated from 35 serum samples of the infected group (B) and of 40 samples of the healthy group (A) dog populations, revealed that significantly ($P < 0.05$) decrease in total serum protein (TP) value for infected group (B) compared with healthy group (A). Similar trend has been reported earlier by Kaymaz *et al.* (1999) and Nwoha *et al.* (2013). Hypoproteinemia could be attributed to the interference with efficacy of digestion and absorption by intestinal mucosa due to nematodal infection (Kumar *et al.*, 2014). In current study, both ALT and AST values were significantly increased ($P < 0.01$) in the infected group and may be a result of damage of liver and from migrating nematodal larvae. Additionally, there was a highly significant increase ($P < 0.01$) in ALP in the infected group compared with the healthy one. Similar result was reported by Willesen *et al.* (2009), however, there is not enough research on ALP changes in dogs infected with intestinal nematodes. This significant increase in ALP in infected dogs could also be attributed to hepatobiliary damage secondary to nematodal larvae migration.

It is worthwhile to mention that significance hematological and biochemical variation in dogs with GI parasitism have been documented in India, Pakistan, Zambia and other countries, however, to the best of our knowledge, this is the first time that an assessment of the health status of dogs infected with intestinal nematodes utilizing data of clinical hematological and biochemical findings has been conducted in Egypt.

In conclusion, intestinal nematode in dogs was common in Upper Egypt with infection rate of 32.5% and *Toxocara canis* was the most common nematode infection (23.3%). They have significant effects on hematological and biochemical parameters suggesting

their importance as a health problem in dogs. Therefore, the study recommends regular deworming and implementation of proper endoparasitic eradication system to avoid the risk of intestinal nematodal infection in dogs and their zoonotic potential.

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دراسة مستقبلية للنمط الإكلينيكي والوبائي لعدوى الديدان الإسطوانية المعوية في الكلاب في صعيد مصر

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أجريت الدراسة للتقييم الإكلينيكي وتحديد النمط الوبائي للديدان الإسطوانية المعوية في الكلاب في صعيد مصر وتأثيرها على مختلف المعايير الدموية والكيميائية الحيوية. أشتملت الدراسة على عدد ١٢٠ كلب (العمر = شهر واحد إلى ٣ سنوات) ، من الجنسين وسلالتين مختلفتين (صغيرة وكبيرة). أجريت الاختبارات الطفيلية والإكلينيكية والدموية والبيوكيميائية والوبائية. أظهر التحليل المجهرى لعينات البراز (n = 120) ثلاثة أنواع من الديدان الإسطوانية المعوية: *Toxascaris* ، *Toxocara canis* (n = 28) ، *leonina* (n = 5) ، *Ancylostoma caninum* وعدوى مختلطة *Toxocara and Dipylidium caninum* (n = 2) مع معدل إصابة كلي ٣٢.٥٪ (١٢٠/٣٩). مثلت *Toxocara canis* النوع الغالب من عدوى الديدان الإسطوانية المعوية في الكلاب (٢٣.٣٪). تأثرت الجراء (٠- > ٣ أشهر) بشكل كبير بالإصابة بالديدان الإسطوانية المعوية (٥٤.٨٪) ، ولا سيما الإصابة بـ *Toxocara canis* (45.2%). أظهرت الجراء المصابة فقدان الشهية ، شاحبة الأغشية المخاطية وانتفاخ البطن والقيء. أظهرت الدراسة مجموعة من الحيوانات (n = 54) بدون علامات سريرية ، ولكن تم الاستدلال على إصابة جزء من هذه المجموعة بالديدان الإسطوانية المعوية (٢٥.٩٪) مما يشير إلى أن غياب الأعراض السريرية لم يكن دليلاً على عدم وجود الإصابة. أظهر الجنس والسلالة اختلافات غير معنوية (P < ٠.٠٥) على معدل الإصابة بالديدان الإسطوانية المعوية ولكن الموسم كان له تأثير كبير على عدوى الديدان الإسطوانية حيث كان معدل الإصابة (P > ٠.٠٥) في الشتاء بنسبة ٤٢.٣٪ والخريف ٤٣.٨٪ مع اعلي معدل للإصابة في ديسمبر (٦٠٪) ويناير (٥٦.٥٪). أظهرت بيانات الدم التي تم الحصول عليها من ٣٥ عينة دم مصابة و ٤٠ عينة من مجموعات الكلاب الصحية انخفاضاً ملحوظاً (P < ٠.٠٥) في بارامترات كرات الدم الحمراء (PCV ، Hb ، RBCs) والصفات الدموية في المجموعة المصابة ، في حين أن الاختلافات بين المجموعات لكرات الدم البيضاء، كانت عالية غير معنوية. أظهرت البيانات البيوكيميائية الناتجة انخفاض معنوي (P < ٠.٠١) في قيمة البروتين الكلي في الدم في المجموعة المصابة. كما أظهرت إنزيمات الكبد زيادة معنوية (P < ٠.٠١) في مصل ALP ، ALT ، AST في المجموعة المصابة. في الختام ، الإصابة بالديدان الإسطوانية المعوية في الكلاب شائعة في صعيد مصر مع معدل إصابة ٣٢.٥٪ كانت *Toxocara canis* الأكثر شيوعاً (٢٣.٣٪) مع تأثير على المؤشرات الدموية والبيوكيميائية مما يشير إلى أهميتها كمشكلة صحية في الكلاب.