Prevalence and Pathology of Some Internal Parasites in Stray Cats (*Felis catus)* in Khartoum North Town, Sudan

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Abstract

The present investigation was conducted to determine the incidence of internal parasites and their related pathological and haematological changes in stray cats in Khartoum North area, Sudan. A total of 50 cats of different sex and age were captured from the back yards of restaurants, hotels, hospitals and streets of residential areas in different parts of the town. They were all examined for the presence of internal parasites by faecal examination and by direct recovery of worms from the gastrointestinal tract at necropsy. The results revealed the presence of two species of cestode parasites namely *Joyeuxiella spp* and *Diplopylidium spp* with a prevalence rate of 60% and. 30% respectively. In addition, there was one nematode species *Physaloptera praeputialis* (34%;), one Acanthocephala *Onicola canis* (4%) and three species of protozoan parasites including *Toxoplasma gondii/ Hammondia hommondi* (56.3%), *Cryptosporidium spp* (3.1%) and *Cystoisospora rivolta* and *Cystoisospora felis* (34.4% and 6.3%, respectively). No significant macroscopic lesions were associated with the presence of these parasites in the gastrointestinal tract of stray cats. However, microscopic evidence of subacute or chronic gastritis, focal erosions and deep ulcerations of the gastric mucosa were observed in stray cats infected with the nematode parasite *Physaloptera praeputialis***.** In addition,

subacute and chronic catarrhal enteritis were further observed with the presence of *Diplopylidium spp*, *Joyeuxiella spp* and *Oncicola canis*. Fragments of cestode parasites were detected in the lumen of the small intestine. No significant changes in haematological parameters including haemoglobin (Hb) concentration, packed cell volume (PCV) and total white cell count (WBCs) in stray cats harbouring the above mentioned parasites.

Keywords: Stray cats - internal parasites - nematodes – cestodes – protozoan parasites

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Introduction

Stray cats, also known as feral cats are the offspring of the domestic cat *Felis catus* that are free roaming in nature and residential areas. They live in different environmental conditions which enable them to be in direct and indirect contact with human beings and other animals. Stray cats are powerful predators; actively consume a wide-range of foodstuff which originates from different kinds of prey animals (Flux, 2007). That is why they are always exposed to various types of infective agents and thereby act as an important reservoir for them. Stray cats are therefore considered a potential hazard for public health as they can harbour a wide range pathogenic organisms including viruses, bacteria, fungi and several species of protozoan and helminthic parasites (Adams, 2003; Adams et al, 2008; Gates *et al.,* 2009; Stojanovic and Foley, 2011). A great deal of concerns have recently increased on the public health hazards of stray cats because a number of parasitic and other potentially zoonotic diseases are associated with their presence in the local environment (Hill *et al.*, 2000; McGlade *et al.,* 2003; Ballweber *et al.,* 2010). It has been reported that several species of enteric protozoan and helminthic parasites of cat are capable of infecting humans and cause serious zoonotic diseases. For example, the protozoan parasite *Cryptosporidium felis* was reported to cause an acute disease in man characterized by diarrhoea (Sargent *et al.*, 1998; Caccio et al, 2002). Also, *Toxoplasma gondii* was found to cause an asymptomatic disease in humans frequently associated with

immunosuppression, abortion and genital infections (Spain *et al.*, 2001; Montoya and Liesenfeld, 2004). Some commonly occurring gastrointestinal nematodes of cats such as the hook worm (*Ancylostoma spp.*) was reported to cause a characteristic skin lesion in man known as creeping eruption or "cutaneous larva migrans" (Bowman *et al.,* 2010). Another round worm such as *Toxocara spp* was also found to infect humans and cause a serious pathological condition known as "visceral larva migrans" in which the migrating larvae invade multiple tissues of the host (Lee *et al.,* 2010 ; Woodhall and Fior, 2014).

Limited information is currently available on the public health hazards of stray cats in the Sudan. The present study was carried out to determine the prevalence of internal parasites in stray cats in a specific residential area within the capital city of the country in order to develop protective measures and future control plans against their threats for human and animal health.

Materials and Methods

Study area

The present study was carried out in Khartoum North town which is one of the three sister towns that form Greater Khartoum City, the national capital city of the Republic of Sudan. The metropolitan area of Greater Khartoum City comprises Khartoum, Omdurman and Khartoum North and lies between 15 and 16 degrees latitude north and between 31 and 32 degrees longitude east. Khartoum North town is located on the north bank of the Blue Nile.

Capturing of cats

A total of 50 adult stray cats (30 males and 20 females) were captured by baited cage traps from the backyard of hospitals, hotels, restaurants and streets of residential areas in different parts of Khartoum North town during the period between October 2017 and December 2018. Each cat was clinically examined and its age and sex were recorded along with the date of examination. The stray cats were then humanely euthanized by an intramuscular injection of a high dose (30mg/Kg. bwt) of 10% ketamine hydrochloride (Zoetis Inc.) followed by chloroform inhalation (Arbabi and Hooshyar, 2009). Blood samples were obtained from individual cats after initial sedation with ketamine just before euthanasia. The blood was directly collected from the heart in

vials containing the anticoagulant ethylene diamine tetraacetic acid (EDTA) and immediately used for haematological investigation. Normal (reference) values of blood parameters of cats were further obtained from healthy home reared pet cats (n=10) after having permission from their owners.

Postmortem Examination and Worm Recovery

Detailed postmortem examination was carried out on each individual cat for detection of internal parasites and their related grossly detectable lesions in the gastrointestinal tract and other visceral organs. Representative specimens of the stomach, intestines, heart, liver, kidneys and brain were also collected and fixed in 10% buffered neutral formalin solution for histopathological examination. All recovered worms were first cleaned with normal saline solution before being identified. The cestode parasites were then fixed in Rouda-bush solution and the nematodes were preserved in alcohol-glycerol mixtures. The recovered worms were then identified and classified by using the taxonomic key of Soulsby (1982).

Faecal Examination

A minimum of 4 g of fresh faecal samples were obtained directly from the rectum and immediately placed into a container filled with 10% formalin solution for parasitological examination by direct faecal smears, formalin-ether sedimentation technique and saturated sugar for floatation techniques followed by microscopy (Soulsby, 1982). Maximum effort was made to identify and classify protozoan parasites and the different types of eggs of helminthic parasites. The preparations were then observed under X10 and X40 magnifications with ocular micrometre. Faecal smears were further prepared and stained with modified and unmodified Ziehl Neelsen techniques for the presence of *Cryptosporidium* oocysts as described by Henricksen and Pohlenz (1981).

Haematological Methods

The methods described by Weiss and Wardrop (2010) were used for the routine haematological investigations of some blood parameters including hemoglobin (Hb) concentration, packed cell volume (PCV), white blood cell count (WBCs) and differential count.

Histopathological Methods

Histopathological processing of tissue samples obtained from stray cats was performed according to the paraffin wax method described by Bancroft and Gamble (2007). The formalin fixed tissue specimens were dehydrated by ascending grades of ethyl alcohol ranging from 70% to absolute alcohol. They were then cleared by xylene and chloroform and impregnated in paraffin wax. The paraffin wax blocks were sectioned at 6 µ thickness by a rotary microtome (Leica RM 2235) and then stained with haematoxylin and eosin (H&E).

Statistical analysis

Statistical analysis was carried out using the statistical computerized programme SPSS (Microsoft ver. 10, USA). Data were analyzed with Student t-Test and correlation coefficient for comparison between means of haematological parameters in stray cats and reference values. Level of significance was taken at $P < 0.05$.

Results

Parasitological Findings

Several types of helminthic and protozoan parasites with variable infection rates were detected in stray cats collected from various locations at Khartoum North area (Figures 1,2,3,4; Tables 1,3). The recovered helminthic parasites included two genera of Cestodes, one genus of Nematodes and one genus of Acanthocephala. The cystode parasites comprised *Joyeuxiella spp* and *Diplopylidium spp* with infection rates of 60% and 30% respectively (Table1). The nematode parasite was identified as *Physaloptera praeputialis* with an infection rate of 34%; The Acanthocephalan parasite was identified as *Oncicola canis* with an infection rate of 4%. The overall infection rate of Cystodes, Nematodes and Acanthocephala were 76%, 34% and 4% respectively (Table 2).

The types of protozoan parasites recovered in stray cats are shown in Table 3. They comprised the following species of coccidian parasites: *Cystoisospora rivolta* and Cystoisospora *felis,*

Toxoplasma gondii / *Hammondia hammondi* and *Cryptosporidium spp* with infection rates of 34.4%, 6.3%, 56.3 and 3.1% respectively.

Infection rate of helminthic and protozoan parasites in different age groups of stray cats is shown in Tables 4and 5. Very young cats of less than one year old were found to have the lowest rate infection as compared to other age groups. On the other hands, relatively older cats of one and up to three years old were shown to have the highest rate of infection with various types of helminthic and protozoan parasites.

Table 1: Incidence of different species of helminthic parasites in stray cats

Helminths	No. of cats examined	No. of cats infected	Infection rate
Joyeuxiella spp	50	30	60%
Diplopylidium spp	50	15	30%
Physaloptera praeputialis	50	17	34%
Onicola canis	50	2	4%

Table 2: Overall infection rate of helminthic parasites in stray cats

Helminth Class	No. of cats examined	No. of cats infected	Infection rate
Cestodes	50	38	76%
Nematodes	50	17	34%
Acanthocephala	50	2	4%

Table 3: Incidence of different species of protozoan parasites in stray cats

Protozoa	No. of cats examined	No. of cats infected	Infection rate
Cystoisospora rivolta	50	11	34.4%
Cystoisospora felis	50	2	6.3%
Toxoplasma/ Hammondia	50	18	56.3%
Cryptosporidium spp	50	1	3.1%

Table 4: Infection rate of helminthic parasites in different age groups of stray cats

Parasites	Age group (Years)		
	< 1	1—3	> 3
Joyeuxiella spp	2(15.4%)	8(61.5%)	3(23.1%)
Diplopylidium spp	0(0.00%)	3(50.0%)	3(50.0%)
Physaloptera praeputialis	1(33.3%)	1(33.3%)	1(33.3%)
Joyeuxiella spp+ Diplopylidium spp	1(20.0%)	3(60.0%)	1(20.0%)
Joyeuxiella spp+ Physaloptera praeputialis	0(0.00%)	1(11.1%)	8(88.9%)
Joyeuxiella spp+ Onicola canis	0(0.00%)	0(0.00%)	1(100%)
Diplopylidium spp+ Physaloptera praeputialis	0(0.00%)	0(0.00%)	2(100%)
Physaloptera praeputialis+ Onicola canis	1(100%)	0(0.00%)	0(0.00%)
Joyeuxiella spp+ Diplopylidium spp+ Physaloptera praeputialis	0(0.00%)	0(0.00%)	2(100%)

Table 5: Infection rate of protozoan parasites in different age groups of stray cats

Parasite	Age group (Years)		
	< 1	1—3	> 3
Cystoisospora rivolta	0(0.00%)	4(66.7%)	2(33.3%)
Toxoplasma/ Hammondia	2(15.4%)	7(53.8%)	4(30.8%)
Cryptosporidium sp	0(0.00%)	0(0.00%)	1(100%)
Cystoisospora rivolta+ Cystoisospora felis	0(0.00%)	0(0.00%)	1(100%)
Toxoplasma/ Hammondia+ Cystoisospora felis	0(0.00%)	0(0.00%)	1(100%)

Table 6: The effect of internal parasites on some haematological values of stray cats

Parameter	Infected Cats	Reference Values*
PCV (%)	35.23±1.47	36.00±4.46
Hb (g/dL)	10.10±0.43	11.04±2.20
WBCs /µL	3.80±0.04	4.2±0.9
Lymphocytes /µL	54.00±1.93	51.42±5.17
Neutrophils /µL	31.95±1.76	36.28±6.03
Monocytes /µL	5.39±0.44	4.71±0.28
Eosinophils /µL	7.79±0.18	7.28±0.89
Basophils /µL	1.09±0.06	1.00±0.00

*No significant differences were observed ($P < 0.05$.)

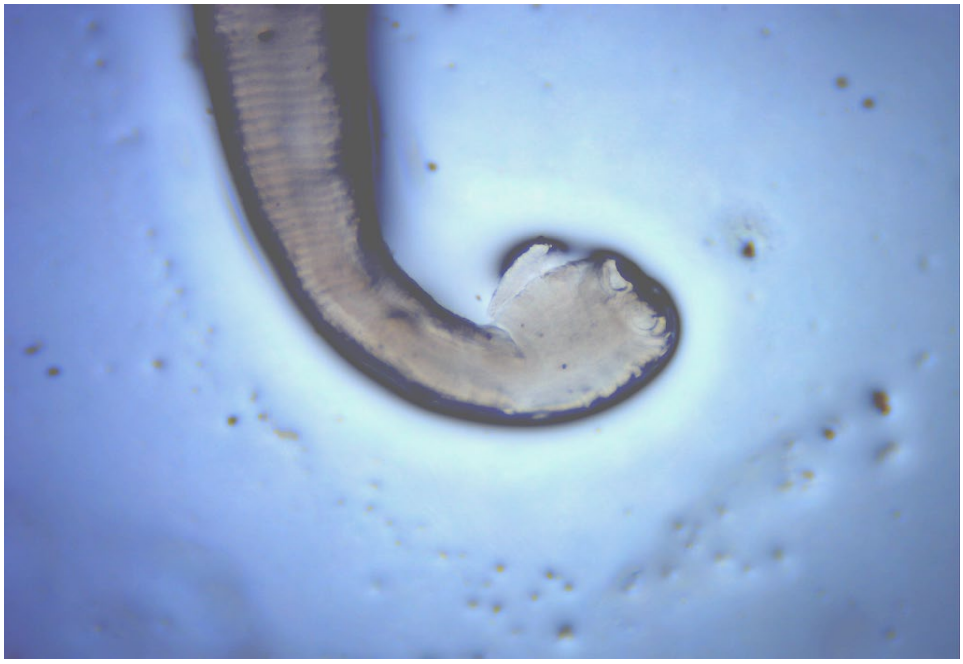

Figure 1: Anterior part of the cat tapeworm *Diplopylidium spp* recovered from the lumen of the small intestine.

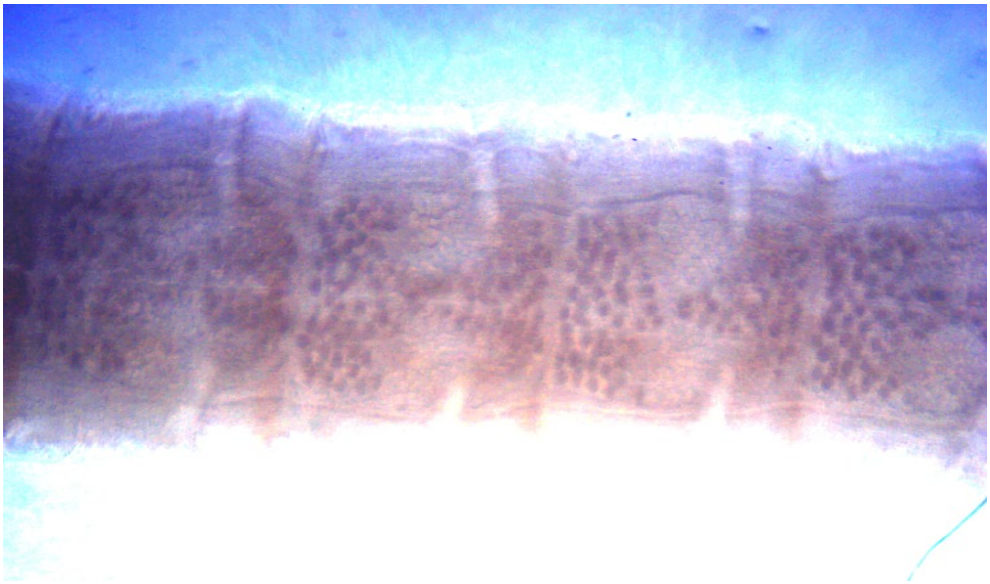

Figure 2 : Fragment of the cat tapeworm *Joyeuxiella spp* recovered from the lumen of the small intestine.

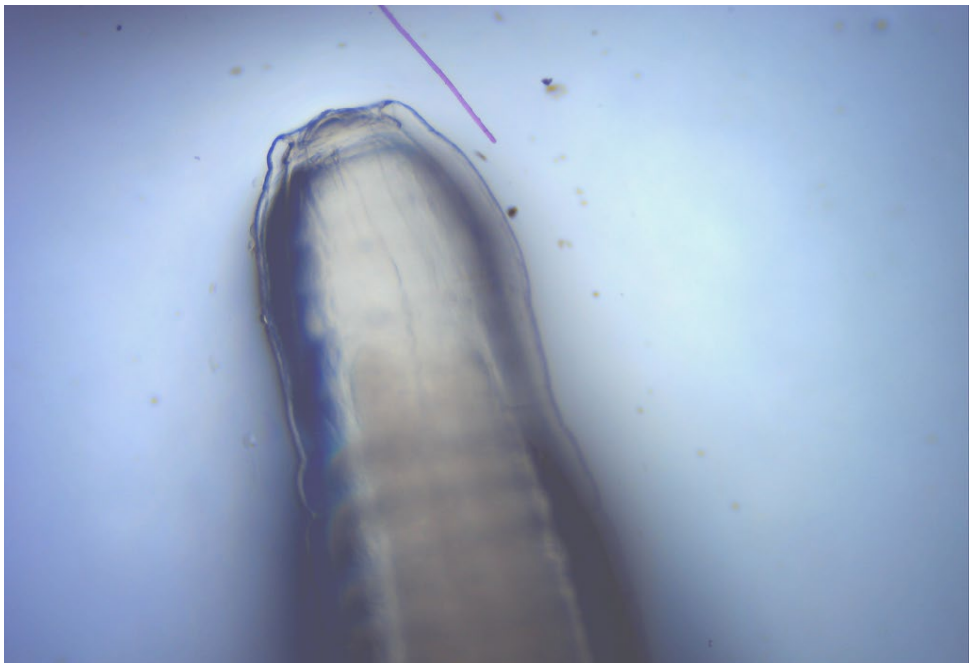

Figure 3 : Anterior part of the cat roundworm *Physaloptera praeputialis* recovered from the lumen of the stomach.

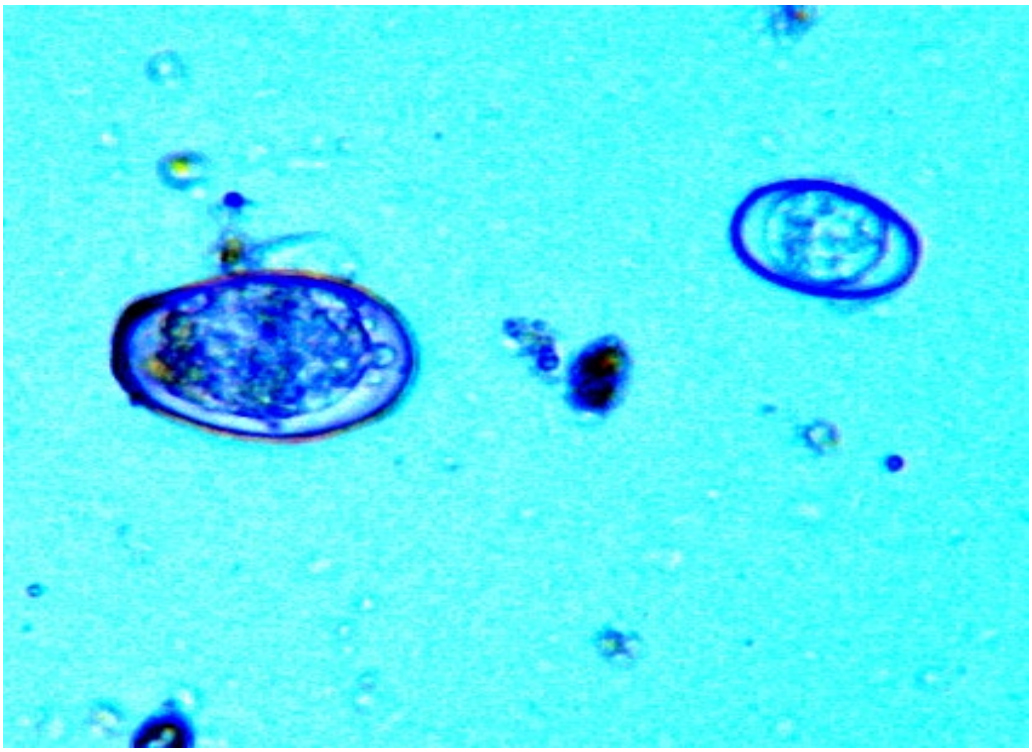

Figure 4 : Oocysts of Cysto*isospora felis* (large size) and *Cystoisospora rivolta* (medium size) photographed from faecal sample (X10).

Figure 5: Small intestine with nematode infection showing oedema and thickening of the intestinal wall.

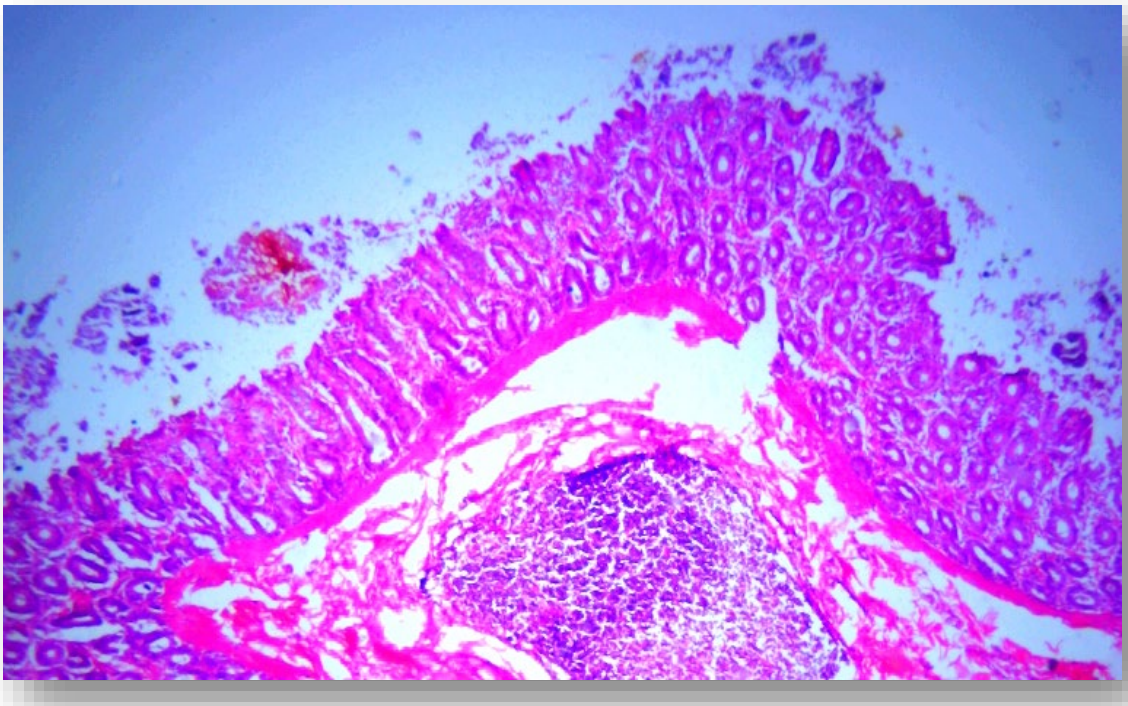

Figure 6: Stomach showing oedema on the submucosa, lymphocytic granuloma and desquamation of the surface epithelium (H&E X100).

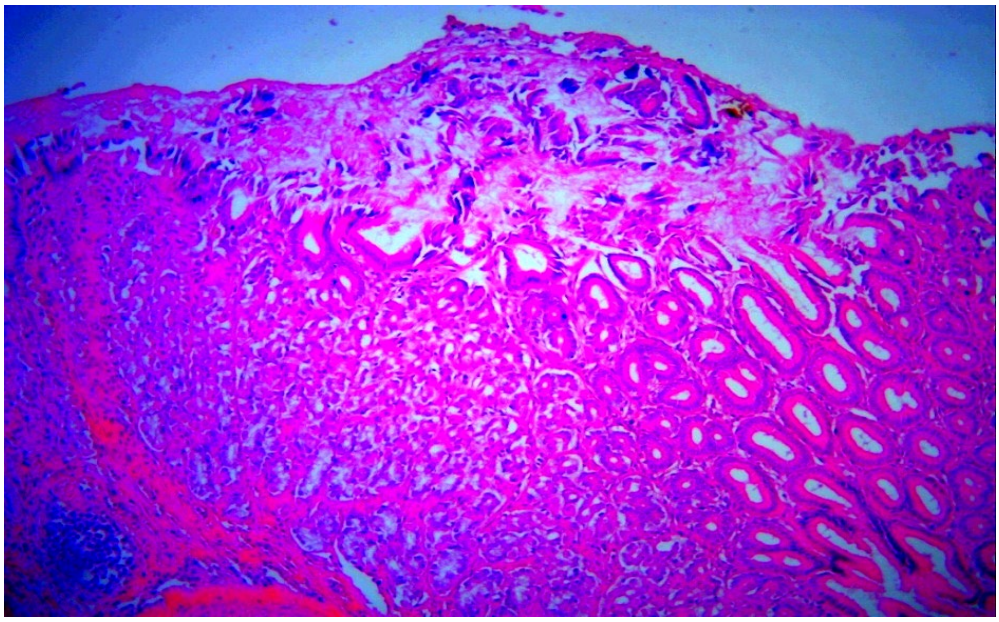

Figure 7: Stomach showing focal necrosis of the superficial areas of the gastric mucosa forming shallow erosions filled with inflammatory exudate (H&E X100).

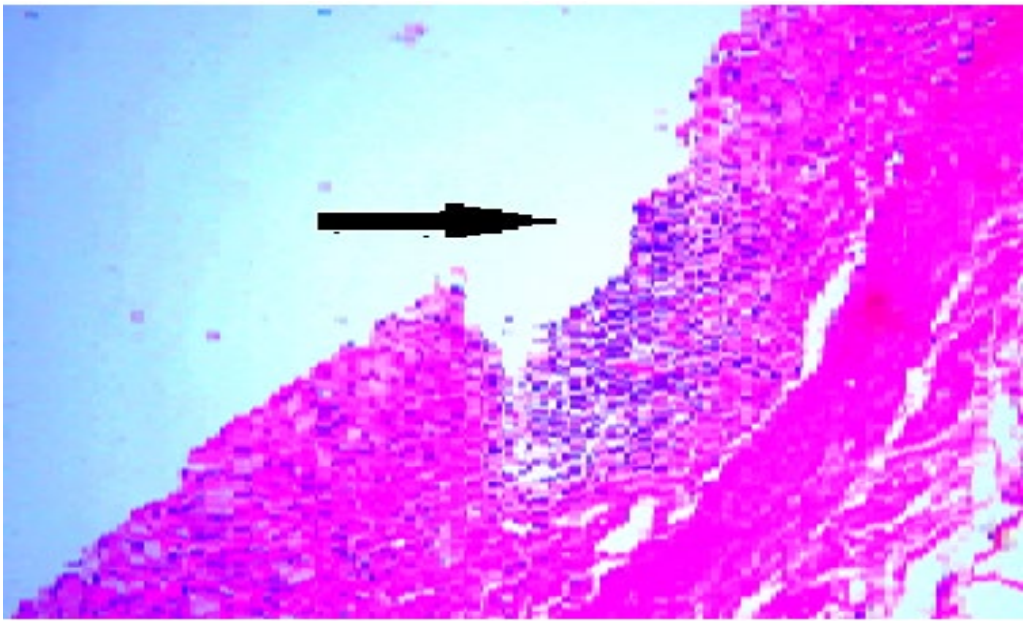

Figure 8: Stomach showing deep ulceration (arrow) with massive lymphocytic infiltration on the damaged part of the gastric mucosa (HE X 100).

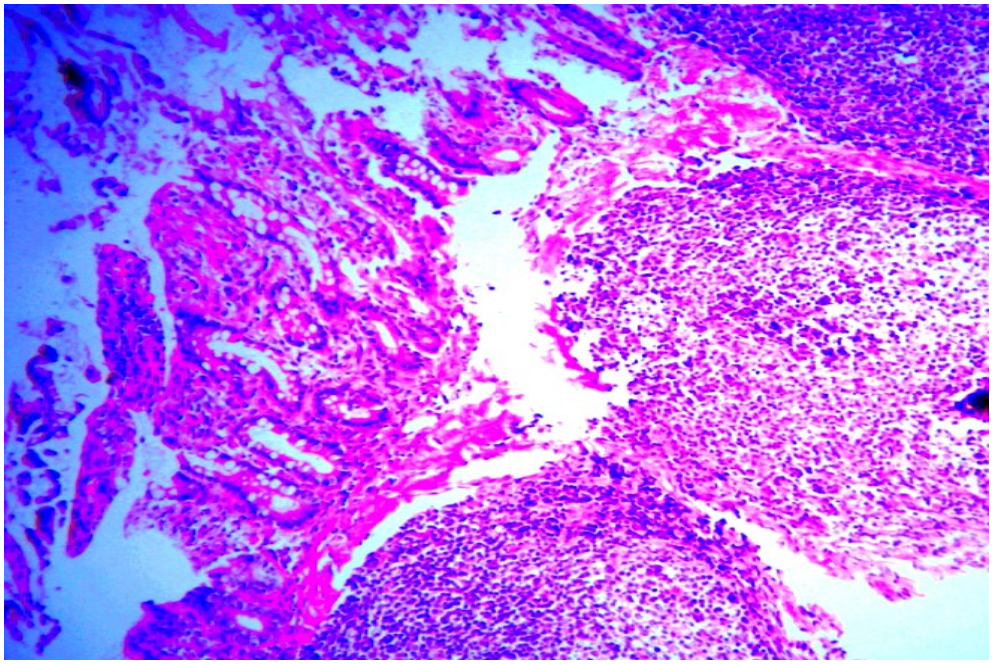

Figure 9: Small intestine showing deposition of mucous exudate, disintegration of intestinal villi and hyperplasia of the gut – associated lymphoid tissue (H&E X100).

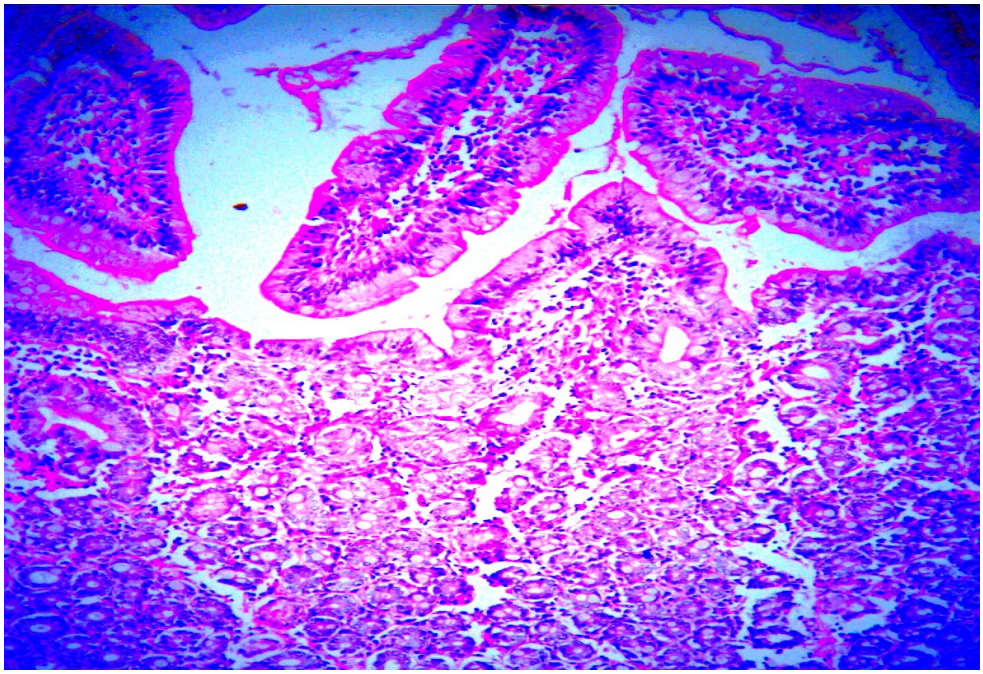

Figure 10: Small intestine showing atrophy of the intestinal villi and mucous glands and diffuse mononuclear cellular infiltration on the lamina propria (H&E X100).

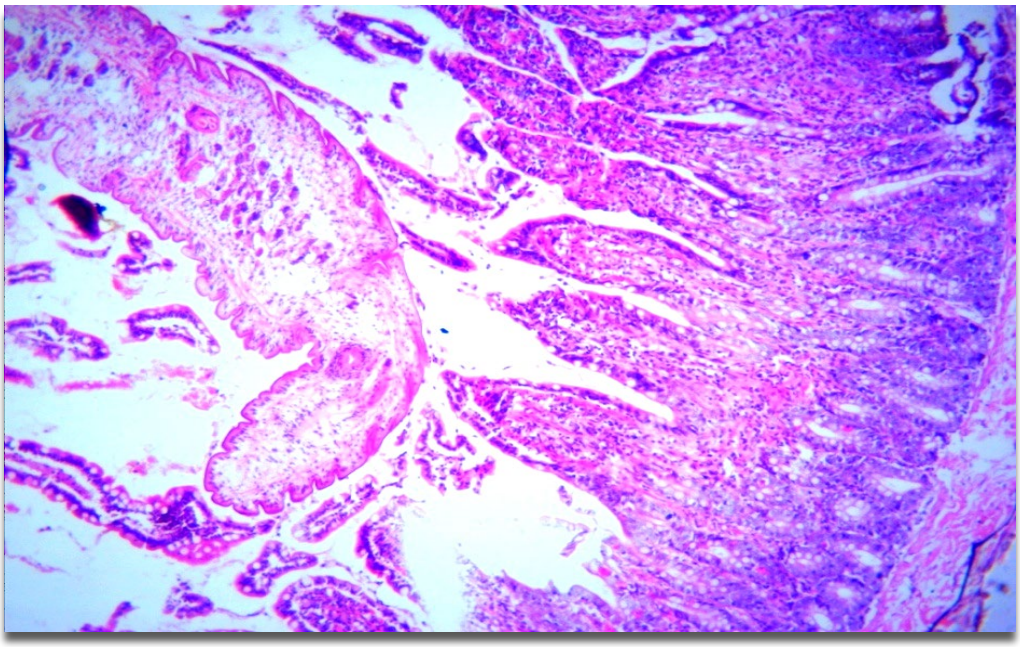

Figure 11: Small intestine showing longitudinal section of a cestode parasite in the lumen of the small intestine (H & E X 100).

Pathological Findings

Gross examination of visceral organs of stray cats infected with different types of helminthic and protozoan parasites did not reveal significant macroscopic lesions other than diffuse congestion of the small intestines, accumulation of mucous exudate and thickening of the intestinal wall (Figure 5). However, the most salient histopathological changes related to the presence of internal parasites were mainly detected in the stomach and small intestines. The stomach showed subacute or chronic gastritis as reflected by diffuse cellular infiltration, oedema, lymphocytic granuloma on the submucosa and desquamation of the lining epithelium of the mucosal surface (Figure 6). These changes were associated with the presence of the nematode parasite *Physaloptera praeputialis* in the lumen of the stomach. Other histopathological changes of the gastric mucosa included focal necrosis of epithelial cells in the superficial areas forming shallow erosions filled with inflammatory exudate (Figure 7). In some other cases, deep ulcerations with

massive infiltration of lymphocytes were also observed on the damaged parts of the gastric mucosa (Figure 8). The histopathological changes of the small intestines were associated with the presence of *Diplopylidium spp, Joyeuxiella spp* and *Oncicola canis*. The affected intestines showed microscopic evidence of subacute or chronic catarrhal enteritis characterized by increased number of active goblet cells and excessive deposition of mucous exudate. In addition, there was diffuse infiltration of mononuclear cells and massive hyperplasia of the gut – associated lymphoid tissue (GALT) forming large discrete lymphoid follicles on the intestinal submucosa (Figure 9). Atrophy of the intestinal villi and diffuse infiltration of lymphocytes and other types of mononuclear cells were also observed in some other cases (Figure 10). Fragments of cestode parasites were occasionally observed in the lumen of the small intestine (Figure 11).

Haematological findings

The effect of internal parasites on some haematological parameters of stray cats is shown in Table 6. There was no significant differences ($P > 0.05$) in the values of haemoglobin (Hb) concentration, packed cell volume (PCV) and total white blood cell count (WBCs) of stray cats infected with various types of internal parasites as compared to control values. No significant changes were also observed in the numerical count of neutrophils, eosinophils, basophils and monocytes. However, a slight non-significant increase in lymphocytes count was observed in infected cats as compared to controls.

Discussion

The result of the present search on internal parasites in Khartoum North area has generally indicated that stray cats were naturally infected with certain species of Cestodes, Nematodes, Acanthocephala and protozoan parasites. The cestode worms represented by *Joyeuxiella spp* and *Diplopylidium spp* were the most frequently encountered helminthic parasites with an infection rate of 76% followed by one species of nematodes (*Physaloptera praeputialis*) with 34% infection rate. In addition, one species of acanthocephalan worms (*Oncicola canis*) was also present, but at an extremely low percentage (4%). The higher prevalence of cestodes in the

currently examined stray cats in Khartoum North area might be attributed to the massive presence of their intermediate hosts such as coprophagous beetles and reptiles. The higher prevalence and infection rate of cestode parasites in stray cats have previously been reported in different locations in the Sudanese capital city of Khartoum (Magda, 1995; Al-Hindy, 1994) and also in Kashan and Mashad in Iran (Arbabi and Hooshyar, 2009; Borji *et al.,* 2011). The relatively lower prevalence of nematode parasites in stray cats as compared to cestodes has already been reported by several authors (Al-Hindy ,1994; Magda ,1995; Schuster *et al.* 2009; Arababi and Hooshyar, 2009 and El-Azazi *et al,* 2016). In addition, the extremely lower prevalence of the Acanthocephalan *Oncicola canis* in stray cats in the present investigation has also been reported by Magda (1995) and Adams (2003). It is worth mentioning that none of the commonly occurring trematode parasites in cats such as *Platynosomum fastosum* (feline liver fluke) or the lung fluke *Paragonimus kellicotti* were detected in the present study. That was probably due to the absence of their first and second intermediate hosts in the study area.

It is obvious from the results of the present investigation that Khartoum North area is apparently endemic with cestode and nematode parasites. The noticeable prevalence of such parasites in the gastrointestinal tract of stray cats is probably due to the massive presence of various types of infected arthropod intermediate hosts such as beetles, cockroaches, crickets, earwigs and grasshoppers in the study (Kalyanasundaram *et al.,* 2018).

The results of the present investigation have also shown that the overall infection rate with protozoan parasites in stray cats was 52%. This percentage is higher than that reported by Schuster *et al*. (2009) and Arababi and Hooshyar (2009) for protozoan parasites in stray cats in Iran. The results of the present study also revealed that the infection rate with *Toxoplasma/ Hammondia* in stray cats was the highest (56.3%), followed by *Cystoisospora rivolta* (34.4%). On the other hand, *Cystoisospora felis* and *Cryptosporidium spp* showed an extremely lower infection rate of 6.3% and 3.1% respectively. Such a high prevalence of *Toxoplasma gondii/ Hammondia hammondi* in stray cats has also been reported in several countries including Iraq (Mahmoud, 2016) and India (Borkataki et al., 2013). The lower prevalence rate of *Cryptosporidium spp* in stray cats observed in the present investigation has also been reported in India and Thailand (Borkataki *et al.,* 2013 ; Koompapong *et al.,* 2014).

The pathological changes associated with the presence of helminthic parasites in stray cats in the present investigation were mainly confined to the stomach and small intestines. Microscopic features of chronic gastritis with erosions and ulceration of the gastric mucosa, oedema and lymphocytic granulomas in the submucosa were observed with the presence of *Physaloptera praeputialis* in the lumen of the stomach. In addition, the intestinal changes associated with the presence of *Diplopylidium spp, Joyeuxiella spp* and *Onicola canis* were mainly dominated by acute, subacute or chronic enteritis and atrophy of intestinal villi. Similar histological findings were also reported in the gastric and intestinal mucosa of stray cats with parasitic burden of different types of nematodes and cestodes (Magda,1995; Naem *et al*, 2006; Elsheikha et al, 2018).

In the present investigation, the major haematological parameters such as haemoglobin concentration (Hb) and packed cell volume (PCV) were not significantly affected in stray cats as compared to reference values. This is probably due to the fact that most examined cats were collected from the backyards of restaurants and hotels where they can find sufficient and nutritive food to eat. In addition, none of protozoan blood parasites such as *Babesia felis* or blood sucking worms such as hookworm (*Ancylostoma spp*) were detected in all examined cats. It was well observed that the total white blood cell (WBCs) count was not significantly affected by the presence of internal parasites in the gastrointestinal tract of stray cats in the present investigation. However, a slight non-significant increase in lymphocyte count was observed and that was probably due to the chronic nature of parasitic infections.

It is therefore concluded that stray cats in Khartoum North area are naturally infected with certain species of enteric helminthic and protozoan parasites without exhibiting abnormal clinical signs indicative of internal parasitism. However, the presence of zoonotic organisms such as *Toxoplasma gondii* constitutes a potential public health hazard for the human populations in the area. Other types of pets particularly dogs will also be affected by the presence of internal parasites in stray cats. A larger scale parasitological and pathological investigation on stray cats is essentially required in this area and in other parts of the country in order to determine the real size of the problem.

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