



## Research article

## Diversity of external and gastrointestinal parasites and the associated clinical, hematological, and biochemical findings in red fox (*Vulpes vulpes*) of Egyptian Wilderness

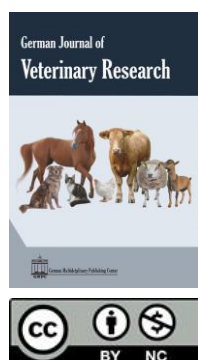
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### Abstract

The red fox (*Vulpes vulpes*) is the most widely distributed and adaptable carnivore in the world. Because they live in the wild, rural, and urbanized communities, red foxes can potentially transmit diseases to animals and humans in different regions. Parasitic infections are a significant and dangerous group of infectious diseases that threaten both animals and humans. There have been very few studies investigating the incidence and health effects of parasitic infections in red foxes. This study was conducted on 44 red foxes living in the Egyptian wilderness to investigate the incidence and health impact of ecto- and endo-parasites. Red foxes of different ages and sexes and from various locations in Egypt and different periods were included in this study. Among all foxes tested, 19 out of 44 (43.2%) were infected by either endo- or ectoparasites, or both, while 25 out of 44 (56.8%) were non-infected. In our study, we found that red foxes are highly affected by *Toxocara canis* (27.3%), *Toxascaris leoninae* (18.2%), and Trematode spp. (4.5%) gastrointestinal parasitic infections. We also observed the presence of different flea species in the examined foxes, including *Ctenocephalidus canis* (25%), *Ct. felis* (18.2%), *Pulex irritans* (6.8%), and *Echidnophaga gallinacea* (4.5%). Additionally, some of the foxes we examined showed *Rhipicephalus sanguineus* (13.6%), suggesting their potential role in transmitting tick-borne diseases. Clinical and biochemical investigation showed that infected foxes had significantly lower levels of total protein and albumin compared to the non-infected group, indicating nutritional disturbances. Furthermore, we observed changes in hematological parameters and cellular immune response in infected (endo- or ectoparasites) and non-infected groups. Specifically, we noted a significant increase in the total leukocyte count and granulocyte percentage, as well as a substantial decrease in hemoglobin levels, in infected animals compared to non-infected ones. This study adds valuable insights to the epidemiology of parasitic diseases, particularly considering the important role that red foxes play in various Egyptian ecological systems.

**Keywords:** Red fox, Tick, Parasite, Hematology, Nematode

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### Introduction

Red foxes (*Vulpes vulpes*) are among the most widespread wild carnivores globally. They are definitive hosts of numerous pathogenic parasites that can affect humans and farm animals. These animals cover large distances while searching for prey and nesting sites,

leading to the spread of parasites across vast geographical areas. Therefore, it is crucial to monitor the parasitic pathogens of red foxes for the sake of public and animal health (Erol et al., 2021). The red fox is adaptable to various environments, including arctic regions, arid temperate deserts, and densely populated cities.

It is the most widely distributed carnivore in the world and the most prevalent carnivore in Egypt (Basuony et al., 2005). In Egypt, the red fox can be found in the Sinai Peninsula, the northern part of the Eastern Desert, the Nile Delta, and the Western Mediterranean Coastal Desert (*Vulpes vulpes aegyptiaca*). It is a large fox with black hair on the back of the ear, a long bushy tail with a white tip, and a large elongated black marking on the foreleg (Osborn and Helmy, 1980).

The red fox has a significant impact on zoonotic medicine and public health in Egypt. In Egypt, rabies in red foxes is a public health concern, according to reports from the World Health Organization (WHO, 1997). Red foxes are known to carry and spread parasites that can negatively affect zoonotic transmission, conservation, and economic value. Understanding red fox parasite infection is crucial due to their importance to the environment (Henderson, 2009). Many parasites of red foxes can also infect native animals, acting as intermediate hosts or reservoir hosts (Thompson et al., 2009). Red foxes can carry parasites such as *Spirometra erinaceieuropaei*, *Toxocara canis*, and *Taenia* spp., which have been found in native wildlife (Spratt et al., 1991). Foxes can also act as reservoir hosts for helminths that can infect domestic and livestock species and easily transmit to domestic dog populations.

An example of this is *T. canis* and *Dipylidium caninum*, which are specifically targeted at canid or carnivore hosts (Gortázar et al., 1998). The red fox is a host for parasites, and its larval forms can infect livestock, including *Echinococcus granulosus* and various *Taenia* species. Controlling parasites in dog populations and helminths in livestock can be challenging due to the presence of the same parasite species in red foxes in the same environments (Richards et al., 1995). Some of the nematodes found in red foxes, such as *T. canis* and *Trichinella spiralis*, as well as cestodes like *E. granulosus*, are medically significant as they cause toxocariasis, trichinellosis, and hydatid disease, respectively (Richards et al., 1995). The risk of zoonotic transmission increases as human activities and environmental factors, such as droughts, bring red foxes into closer contact with human habitats or urban areas. Environmental and soil contamination with parasite eggs is a major

concern, as is the lack of public awareness of the risk of infection from parasites carried by red foxes (Wolfe et al., 2001).

In previous studies, various hematological and biochemical parameters were examined in red foxes (*Vulpes vulpes*). The average red blood cell count was found to be  $5.9 \times 10^{12}/L$ , hemoglobin level was 11.5 g/dl, packed cell volume was 33%, platelet count was  $205 \times 10^9/L$ , and total leucocyte count was  $7.4 \times 10^9/L$  (Hawkey, 2017). Additionally, biochemical parameters were analyzed, with mean values of total protein and albumin determined as 5.2-6.9 g/dl and 2.4-3.6 g/dl, respectively (Lumsden et al., 1979). Furthermore, total protein, albumin, and the albumin/globulin ratio were measured as 7.3 g/dl, 3.4 g/dl, and 0.86 (Rui et al., 2011).

However, there is a lack of data on the prevalence and health effects of parasitic infections in red foxes in Egypt. Consequently, a recent study was carried out on 44 red foxes residing in the Egyptian wilderness, with significant exposure to both rural and urban areas. This study aimed to investigate the occurrence and health impact of ecto- and endoparasites in red foxes of different ages and sexes and from various locations in Egypt, including the governorates of Giza, Sohag, and Qena.

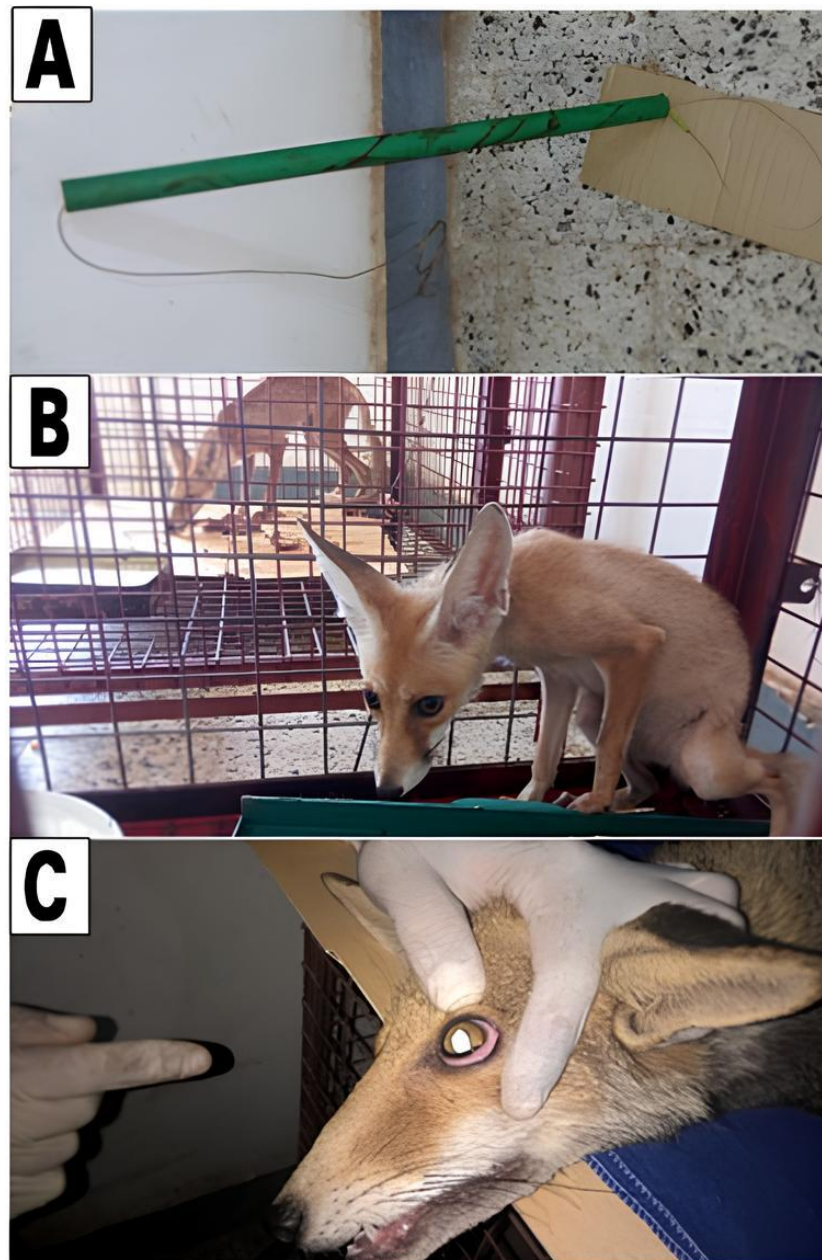
## Materials and methods

### Ethical statement

This research has obtained the approval of the Research BioEthics Committee (RBC) of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt, "VM/SVU/23(1)-01." All efforts were made to minimize the painful procedures and experiments on the hunted foxes used in the current study, following international, national, and institutional guidelines for *in vivo* animal experimentations.

### Geographic location and experimental animals

Animals were hunted from agricultural areas in three governorates in Egypt: Giza in the northern region, Sohag and Qena in the southern region. The study was conducted between February 2016 and April 2021 and focused on 44 red foxes of different ages, sexes, and locations. Foxes were not captured during the winter months, which included gestation and parturition periods, in order to avoid stress and



**Figure 1:** Restraint, housing, and clinical examination of red foxes. (A) Typical dog grasper with a quick-release noose used to catch a fox (Stocker, 2005). (B) Metal pens were designed to best housing of foxes during the study. (C) Inspection of conjunctiva and other mucous membranes.

potential injury to pups and adults. Additionally, no samples were taken during these periods to prevent the measurement of pregnant females and obtaining biased results (Mohamed and Mohamed, 2022).

Animals were captured using foot-held traps baited with tuna or chicken flesh and were checked every six hours to ensure the foxes' safety during the trapping process. When foxes were caught, a hand-held circular snare was used to immobilize them while they were being transported in metal enclosures (Figure 1) (Acosta-Jamett et al., 2010). The sex of the foxes was determined by examining their external

genitalia (22 females and 22 males). Their weight was measured using a digital scale and ranged from 0.7 to 5.4 kg (Slavica et al., 2011). According to Osborn and Helmy (1980), the animals were identified as belonging to the subspecies *Vulpes vulpes aegyptiaca*, characterized by large, black ears and a long, bushy, club-shaped tail with a white tip. They have reddish to reddish brown fur on their ventral, lateral, and ventral sides and large, elongated, black markings on their forelegs.

#### **Clinical inspection**

In both clinically healthy and infected foxes, various clinical parameters were investigated.



This included auscultation of the chest, detection of pulse rate, and measurement of body temperature as previously described (Constable et al., 2017). A complete clinical examination was conducted on each animal, which involved visual inspection of the skin, teeth, all mucous membranes, ears, footpads, and toes, as well as external palpation of the limbs, neck, abdominal organs, and lymph nodes, and abdominal and thoracic auscultation (Curi and Talamoni, 2006).

## **Hematological analysis**

### ***Blood collection***

Foxes were fasted overnight prior to blood collection. Blood samples were collected from the cephalic veins using a syringe with a 21- or 22-gauge needle. Two mL blood samples were collected in vacutainer blood collection tubes containing EDTA and used for hematological examination. Following previous procedures by Rui et al. (2011), hematological investigations were performed on whole blood using a hematology analyzer (Abacus 380, Diatron MI PLC, Hungary).

### ***Biochemistry***

For the serum biochemical evaluation, 3 mL blood samples were centrifuged at 3,000 rpm for 10 min within 1 h of collection. Sera were stored at -80°C in a freezer prior to analysis. Following Rui et al. (2011) and Korhonen and Huuki (2014), the serum biochemical parameters were evaluated using an automatic analyzer (ARX-199 I-SR.NOL 1912534, Micro Lab Instruments, India).

### ***External parasites examination***

The foxes were thoroughly examined for ectoparasite infection. This involved using skin scraping, otic swabs, and combing the entire body with a fine-toothed stainless-steel comb (Zakson et al., 1995). The skin of all animals was palpated and visually inspected for the presence of ticks. Any ticks found were carefully removed to ensure the mouthparts remained intact. Additionally, any fleas and lice found were collected using the comb.

The samples obtained, including ticks, fleas, or scrapings, were collected and placed in plastic bags and then stored in 70% ethanol until processing. After preservation in alcohol, the samples were cleaned with water and then immersed in 5% potassium hydroxide (KOH)

with slight warming for 10-15 minutes to adjust the pH of the samples. The samples were then dried using a series of alcohol concentrations (50%, 70%, 90%, and absolute) for 1-2 hours each. Following this, the samples were placed in xylene twice for 5 minutes each to achieve transparency. They were then mounted using a mixture of styrene, a plasticizer, and xylene and left to dry before examination. Examination of ectoparasites was carried out under a microscope using a magnification of  $\times 4$  to  $\times 10$ , in accordance with species identification based on microscopic examination as described by Soulsby (1982) and Wall and Shearer (2001).

## **Internal parasites examination**

### ***Fecal examination***

Approximately 50-100g of feces from each fox was examined macroscopically for the presence of adult worms and microscopically for the detection of enteric protozoal oocysts with helminth eggs or larvae.

### ***Direct fecal smear***

A small amount (1 g) of fresh fecal sample was placed on a clean slide and mixed with a few drops (0.2 mL) of physiological saline until a smooth suspension was obtained. The mixture was then covered with a slip and examined under a microscope, following the method described by Soulsby (1982).

### ***Concentration sedimentation method***

About 1-5 g of feces was mixed with 30 ml of water and strained through a tea sieve to remove coarse fecal materials. The mixture was then centrifuged at 1,500 rpm for 5 minutes. Then, the sediment was examined as previously described (Soulsby, 1982).

### ***Concentration flotation method***

To check for parasites in fecal samples, 1 mL of sieved fecal samples mixed with water should be examined using the flotation technique. This method relies on the specific gravity of flotation liquids. To carry out the procedure, dilute the sieved samples with 10-20 ml of saturated salt solution in a test tube, filling it to the top. Then, place a cover glass over the top of the tube to make contact with the liquid. After allowing it to sit for 5-10 minutes, gently remove the cover glass and examine the contents under a microscope, following the guidelines outlined by Soulsby (1982).

## Animal euthanasia

All tested animals (n=44) were euthanatized initially by I/M injection of a toxic dose of Ketamine hydrochloride (2mg/kg), then intravenous injection of magnesium sulphate (80mg/kg) according to [Close et al. \(1996\)](#). After verification of complete loss of consciousness, the abdominal cavity of the animal was opened, and the gastrointestinal tract was sectioned.

## Examination of enteric protozoa

For the detection of enteric protozoa, fecal samples, intestinal contents, and mucosal scrapings from different parts of the small and large intestine were obtained. Samples were emulsified in water, strained through a wire mesh screen, and then passed through a double layer of gauze to remove debris.

## Collection of gastrointestinal helminths

The gastrointestinal tract (GIT) was opened along its entire length and searched for mature and immature helminths, both in the contents of the gut and in scrapings of the mucosa. Only nematodes were found during the investigation. The worms were inactivated and fixed in a hot mixture of alcohol and glycerin (70% ethyl alcohol containing 5% glycerine). For the preparation of fixed mounts, the worms were transferred to lactophenol solution and mounted in gelatin glycerin, according to [Belding \(1965\)](#). The collected nematodes and other parasite species were identified as previously described ([Urquhart et al., 1996](#); [Foreyt et al., 2001](#)).

## Statistical analysis

For the apparently healthy animals without parasitic infection, the mean and standard errors for the mean were calculated for each parameter. Data processing and statistical analysis were performed using PRISM software (SAS Institute Inc., Cary, NC, USA). A level of  $p < 0.05$  was accepted as statistically significant.

## Results

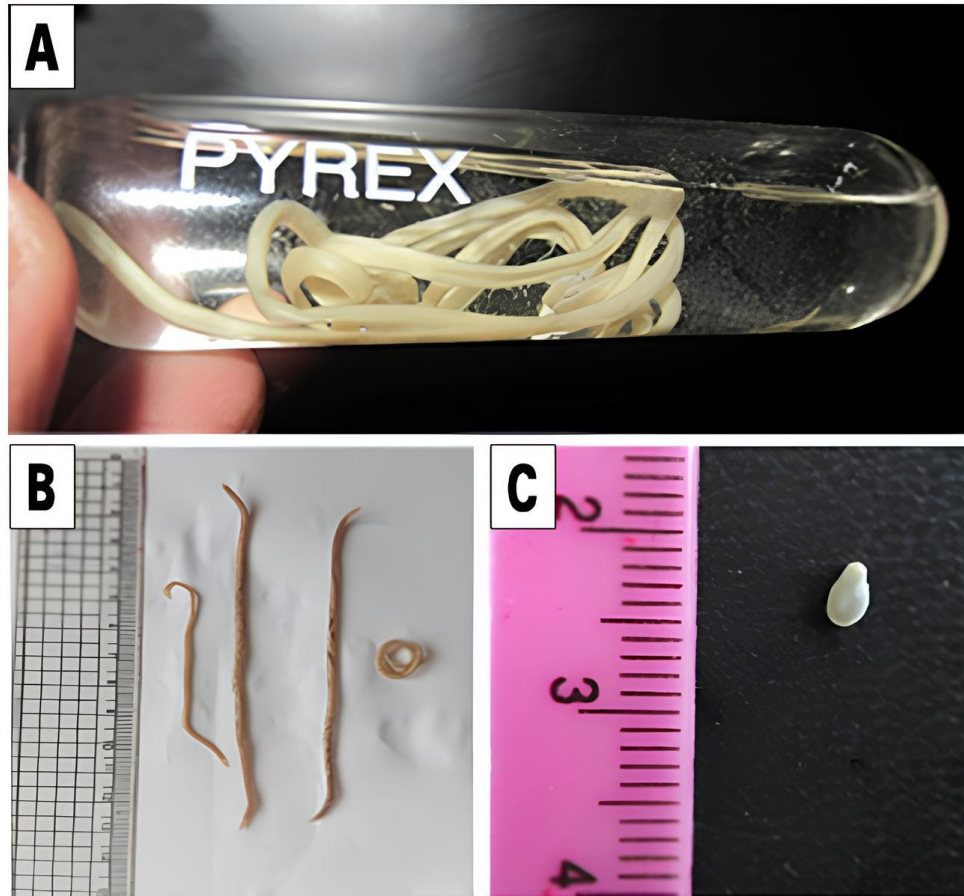
### Parasite identification, infection rate, risk factor assessment

Data from 44 red foxes (*Vulpes vulpes*) were collected, with 22 females and 22 males. Parasitological examination showed that 27.3% (12/44, 95% CI; 15.5-43) of the foxes were infected with at least one internal parasite ([Table 1](#)). The study identified three different species of internal parasites: *T. canis* (27.3%, 95% CI; 15.5-43), *T. leoninae* (18.2%, 95% CI; 8.7-33.2), and a Trematode species (4.5%, 95% CI; 0.8-16.7) ([Table 1](#), [figures 2](#) and [3](#)). The identified ectoparasites (5 species) were found in 34.1% of the examined red foxes, including 4 species of fleas and one species of ticks. The single infestation of external parasites in the examined red foxes revealed the prevalence of *Ct. canis* (25%, 95% CI; 13.7-40.7), *Ct. felis* (18.2%, 95% CI; 8.7-33.2), *P. irritans* (6.8%, 95% CI; 1.8-19.7), and *E. gallinacea* (4.5%, 95% CI; 0.8-16.7). In the case of ticks, *R. sanguinatus* was observed in 6 out of 44 foxes (13.6%, 95% CI; 5.7-28.1) ([Table 1](#), [figures 4](#) and [5](#)).

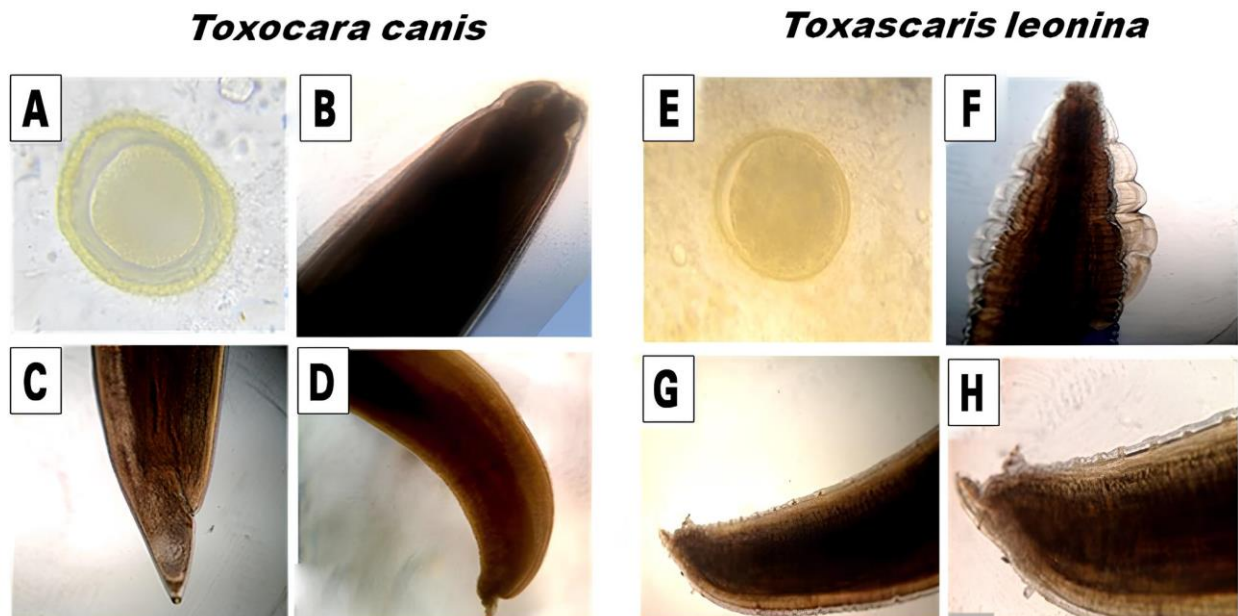
**Table 1:** Prevalence of parasites (all endo- and ectoparasites) species of red foxes (*Vulpes vulpes*) from different localities in Egypt (n=44).

Parasite	No. of negative (%)	No. of positive (%)	95% CI*
<i>Toxocara canis</i>	32 (72.7)	12 (27.3)	15.5-43
<i>Toxascaris leoninae</i>	36 (81.8)	8 (18.2)	8.7-33.2
All nematodes infection	32 (72.7)	12 (27.3)	15.5-43
<i>Trematode sp.</i>	42 (95.5)	2 (4.5)	0.8-16.7
All trematodes infection	42 (95.5)	2 (4.5)	0.8-16.7
All Endoparasites species	32 (72.7)	12 (27.3)	15.5-43
<i>Ctenocephalidis canis</i>	33 (75)	11 (25)	13.7-40.7
<i>Ctenocephalidis felis</i>	36 (81.8)	8 (18.2)	8.7-33.2
<i>Pulex irritans</i>	41 (93.2)	3 (6.8)	1.8-19.7
<i>Echidnophaga gallinacean</i>	42 (95.5)	2 (4.5)	0.8-16.7
All flea sp. Infection	29 (65.9)	15 (34.1)	20.9-50
<i>Ripicephalus sanguinatus</i>	38 (86.4)	6 (13.6)	5.7-28.1
All tick sp. Infection	38 (86.4)	6 (13.6)	5.7-28.1
All ectoparasites species	29 (65.9)	15 (34.1)	20.9-50

\* 95% CI calculated according to the method described at <http://vassarstats.net/> accessed date 20-24 December 2022

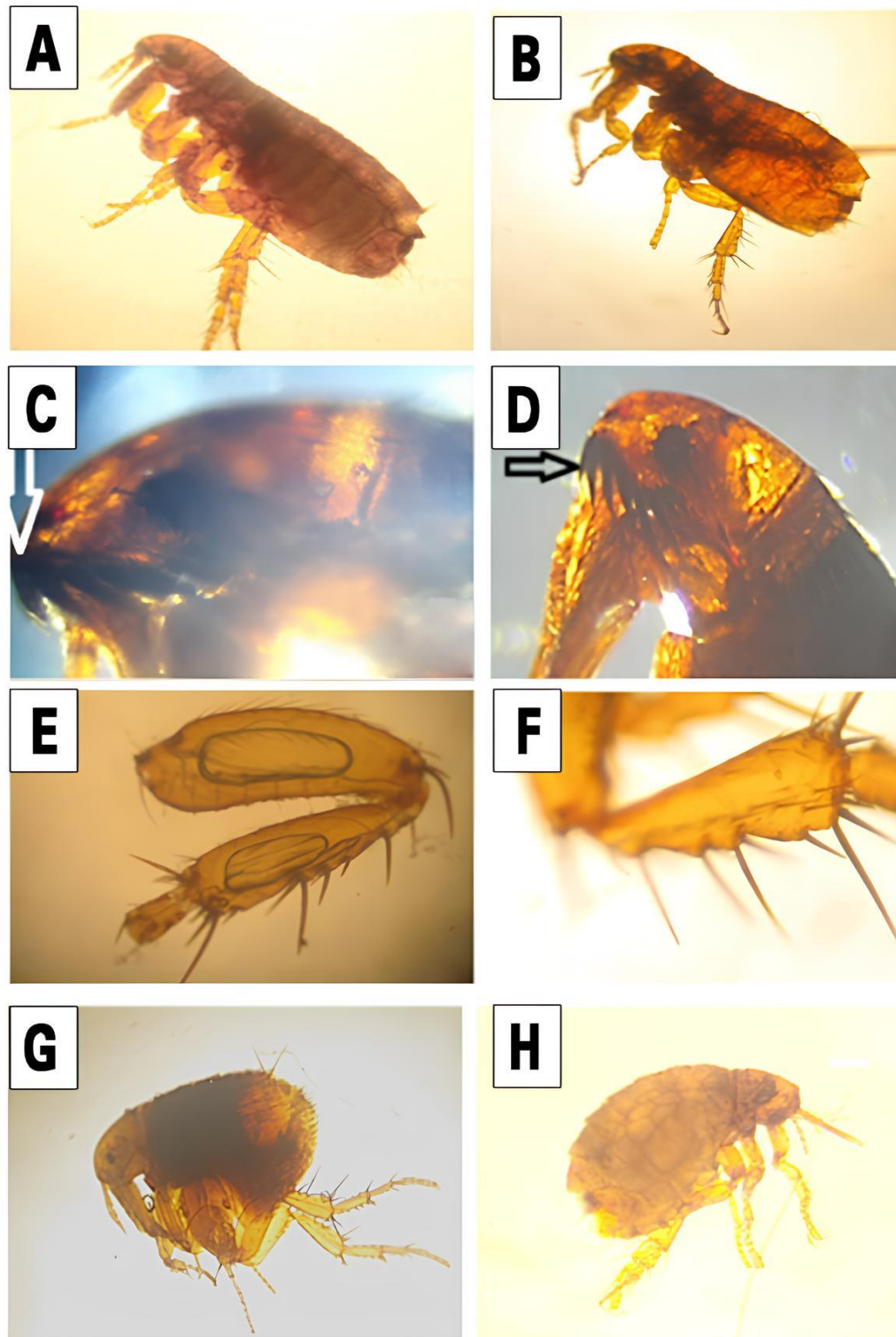


**Figure 2:** Macroscopic examination of trematode and nematode species. A, B, and C: Adult nematodes recovered from examined red foxes. D: Adult trematode species recovered from tested red foxes.

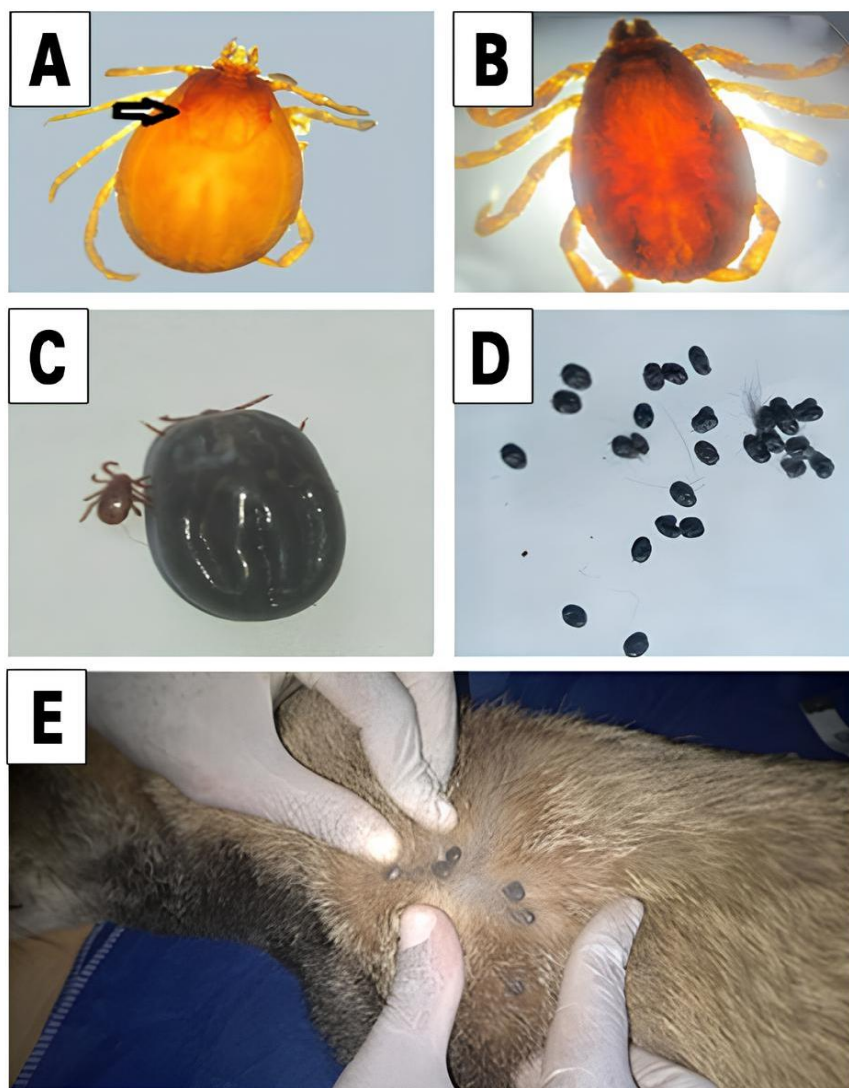


**Figure 3:** Morphology of identified internal parasites. Left panel: A) Egg of *Toxocara canis* showing spherical shape, thick and corrugated eggshell; B) Adult *T. canis* showing anterior end with cervical alae and lips; C) Adult female *T. canis* showing posterior end with pointed end; D) Adult male *T. canis* showing posterior end with finger-like process. Right panel: E) Egg of *Toxascaris leonina* showing a slightly oval shape and smooth eggshell; F) Adult *T. leonina* anterior end shows cervical alae and lips; G and H) Adult *T. leonina* male posterior end shows spicules, finger-like process, and caudal papillae.





**Figure 4:** Various identified flea species in investigated foxes. A) Adult *Ctenocephalidis felis* (×4) showing head is twice as long as high and pointed anteriorly; B) Adult *Ctenocephalidis canis* (×4) showing is more rounded than *C. felis*; C) Closeup of the head of *Ctenocephalidis felis* (×10) and an arrow showing the same length of 1<sup>st</sup> and 2<sup>nd</sup> spines of the genal comb; D) Closeup of the head of *Ctenocephalidis canis* (×10) and an arrow showing 1<sup>st</sup> spine of the genal comb is half as long as 2<sup>nd</sup> spine; E) Close up of tibia of *Ctenocephalidis canis* (×10) showing tibia of all 6 legs have 4-5 teeth; F) Close up of tibia of *Ctenocephalidis felis* (×10) showing tibia of all 6 legs have 7-8 teeth; G) Adult *Pulex irritans* (×4) showing no genal nor pronotal combs; the outer margin of the head is smoothly rounded; H) Adult *Echidnophaga gallinacean* (×4) showing sharply angled head, no genal or pronotal combs.



**Figure 5:** *Ripicephalus sanguineus* adult ticks showing: A) Adult female *R. sanguineus* (×4) showing marked larger than male, scutum covers only the anterior dorsal surface, hexagonal shape of basic capiculi; B) Adult male *R. sanguineus* (×4) showing small size in comparison with female, scutum covers the entire dorsal surface, hexagonal shape of basic capiculi; C) Unengorged or flat (left) and blood-fed engorged (right), *R. sanguineus* Latreille nymphs. The increased size results from ingesting a large volume of blood. D) Adult female *R. sanguineus* engorged with blood; E) Ticks engorged with blood on the skin of adult red fox.

During the examination of parasitic infections in 44 tested foxes, nine double and four triple mixed types of infection were recorded (Table 2). The highest rate of mixed infection was reported with two parasites: *T. canis* with *Ct. canis* or *Ct. felis* (11.4%, 95% CI; 4.3-25.4), followed by *T. canis* with *R. sanguineus* and *T. leoninae* with *Ct. felis* (9.1%, 95% CI; 3-22.6). Additionally, high infection rates were recorded in the case of triple mixed infections: *T. canis* with *T. leoninae* and *Ct. felis* (9.1%, 95% CI; 3-22.6), *T. canis* with *T. leoninae* and *R. sanguineus* (6.8%, 95% CI; 1.8-19.7), and *T. canis* with *T. leoninae* and *E. gallinacean* (4.5%, 95% CI; 0.8-16.7) (refer to Table 2).

Prevalence of infection with at least one internal parasite was the highest in fox samples from the Giza governorate (38.5%), followed by samples from the Qena governorate (33.3%), while animals from Sohag were much lower (20%), although such differences were not statistically significant ( $p > 0.05$ ) (Table 3). Also, the infection rate with internal parasites (at least one parasite) in adult foxes (36 %) was much higher than in young foxes (15.8%), while in female foxes (36.4 %) was higher than males (18.2 %), but no significant differences were recorded ( $p > 0.05$ ) (Table 3). Regarding the rate of infestation with external parasites (at least one parasite), it was the highest in fox samples from the Qena governorate (50%), followed by those



from the Sohag governorate (32%), while animals from the Giza governorate were the lowest (30.8%). Additionally, the rate of infestation with external parasites (at least one parasite) in adult foxes (40%) was much higher than in young foxes (26.3%), while in female foxes (36.4 %) was

higher than in males (31.8%) and these results were illustrated in Table 3. However, such differences in infestation rates of external parasites were also not statistically significant ( $p>0.05$ ).

**Table 2:** Mixed infections of endo- and ectoparasites in Egyptian red foxes (*Vulpes vulpes*).

Type of infection	No. of negative (%)	No. of positive (%)	95% CI*
<i>T. canis</i> + <i>Ct. canis</i>	39 (88.6)	5 (11.4)	4.3-25.4
<i>T. canis</i> + <i>Ct. felis</i>	39 (88.6)	5 (11.4)	4.3-25.4
<i>T. canis</i> + <i>P. irritans</i>	43 (97.7)	1 (2.3)	0.1-13.5
<i>T. canis</i> + <i>E. gallinacea</i>	42 (95.5)	2 (4.5)	0.8-16.7
<i>T. canis</i> + <i>R. sanguinatus</i>	40 (90.9)	4 (9.1)	3-22.6
<i>T. leoninae</i> + <i>Ct. canis</i>	41 (93.2)	3 (6.8)	1.8-19.7
<i>T. leoninae</i> + <i>Ct. felis</i>	40 (90.9)	4 (9.1)	3-22.6
<i>T. leoninae</i> + <i>E. gallinacea</i>	42 (95.5)	2 (4.5)	0.8-16.7
<i>T. leoninae</i> + <i>R. sanguinatus</i>	41 (93.2)	3 (6.8)	1.8-19.7
<i>T. canis</i> + <i>T. leoninae</i> + <i>Ct. canis</i>	42 (95.5)	2 (4.5)	0.8-16.7
<i>T. canis</i> + <i>T. leoninae</i> + <i>Ct. felis</i>	40 (90.9)	4 (9.1)	3-22.6
<i>T. canis</i> + <i>T. leoninae</i> + <i>E. gallinacea</i>	42 (95.5)	2 (4.5)	0.8-16.7
<i>T. canis</i> + <i>T. leoninae</i> + <i>R. sanguinatus</i>	41 (93.2)	3 (6.8)	1.8-19.7

\* 95% CI calculated according to the method described at <http://vassarstats.net/>, accessed date 20–24 December 2022

**Table 3:** Risk factors analysis of parasitic infection in tested red foxes (*Vulpes vulpes*) in Egypt (n = 44).

Analyzed factor	No. of tested	Endoparasites (at least one parasite)			Ectoparasites (at least one parasite)		
		No. of positive (%)	OR (95% CI) #	p value*	No. of positive (%)	OR (95% CI) #	p-value*
<b>Locality</b>							
Sohag	25	5 (20)	Ref	Ref	8 (32)	1 (0.2-4.5)	1.00
Giza	13	5 (38.5)	2.5 (0.6-11.1)	0.263	4 (30.8)	Ref	Ref
Qena	6	2 (33.3)	2 (0.3-14.2)	0.596	3 (50)	2.2 (0.3-16.4)	0.617
<b>Age</b>							
Young	19	3 (15.8)	Ref	Ref	5 (26.3)	Ref	Ref
Adult	25	9 (36)	3 (0.6-13.2)	0.181	10 (40)	1.9 (0.5-6.8)	0.522
<b>Gender</b>							
Female	22	8 (36.4)	Ref	Ref	8 (36.4)	Ref	Ref
Male	22	4 (18.2)	0.4 (0.1-1.6)	0.310	7 (31.8)	0.8 (0.2-2.8)	1.00

# Odds ratio at 95% confidence interval as calculated by <http://vassarstats.net/> (access time, 20-24 December 2022).

\* p value was evaluated by Fisher's exact probability test (two-tailed). Ref; value used as a reference.

### Effect of endoparasite and/or ectoparasite infections on some clinical and laboratory parameters in red foxes

In this experiment, clinical, hematological, and biochemical parameters were analyzed in two groups of animals: one infected with internal or external parasites and another referred to as the control group. In each assessment, whether for internal or external parasites, health impact evaluation, as well as similarity in age, sex, and clinical traits, were considered in selecting the animals. Regarding internal parasite infection, the results showed that the rectal temperature in the control group ( $38.74 \pm 0.06^\circ\text{C}$ ) was very similar to the infected group ( $38.77 \pm 0.07^\circ\text{C}$ ), and the pulse rate (pulse/1 min) of the control group (101.8) versus infected group (113) showed no significant difference ( $p>0.05$ ). However, the

respiratory rate (breath/1 min) was statistically higher in the infected group (27.3) than in the control group (24.6) ( $p=0.0267$ ) (see Table 4). Furthermore, significant decreases were recorded in the levels of estimated glucose, total protein, and albumin in the infected group compared to the control group ( $p<0.05$ ). Additionally, the cellular immune response in the blood revealed a significant increase in the granulocyte number and percentage in the infected group compared to the control group ( $p<0.05$ ). These results demonstrate the adverse health effects induced by internal parasite infection in red foxes (Table 4).

In the case of external parasite infection, the results indicated a similar impact to those infected with internal parasites in terms of a nonsignificant increase in pulse and a significant

**Table 4:** Effect of endoparasites infection on some clinical and laboratory parameters in red foxes (Adult female animals).

Tested parameters (unit)	Control (n=5)		Internally infected (n=3)		p-value
	Mean	SE	Mean	SE	
Temperature (Celsius degree)	38.74	0.06	38.77	0.07	1
Pulse rate (pulse/1 min)	101.8	3.56	113	11.93	0.302
Respiratory rate (breath/1 min)	24.6	0.51	27.3	0.88	0.026
TLC (10 <sup>3</sup> cells/mm <sup>3</sup> )	10.4	1.93	15.7	5.32	0.077
LYM (10 <sup>3</sup> cells/mm <sup>3</sup> )	3.9	0.73	4.7	1.75	0.437
MID (10 <sup>3</sup> cells/mm <sup>3</sup> )	1.2	0.29	2	0.67	0.114
GRAN (10 <sup>3</sup> cells/mm <sup>3</sup> )	5.3	1.03	9	2.97	0.035
LYM%	37.5	7.38	29.3	8.91	0.139
MID%	11.9	2.63	12.8	3.97	0.744
GRAN%	50.6	9.40	57.9	16.78	0.047
RBCs (10 <sup>6</sup> cells/mm <sup>3</sup> )	8.4	1.62	9.3	2.75	0.373
HG (g/dl)	14.1	2.64	12	3.56	0.115
HCT (%)	41.5	7.72	38.2	11.43	0.376
MCV (fl)	50.5	10.31	41.6	12.65	0.294
MCH (pg)	17.2	3.55	13.2	4.02	0.196
MCHC (%)	33.9	6.22	31.6	9.15	0.058
Urea (mg/dl)	92.6	19.21	87.7	27.50	0.738
Creatinine (mg/dl)	0.8	0.19	0.7	0.35	0.513
ALT (U/l)	224.6	65.79	451.7	177.65	0.055
AST (U/l)	197.8	59.49	375.7	145.72	0.075
Glucose (mg/dl)	171.6	32.33	108.7	38.23	0.003
Total proteins (g/dl)	7.6	1.39	6.4	2.13	0.005
Albumin (g/dl)	4.7	0.88	3.9	1.13	0.038
A/G ratio (numerical)	1.2	0.26	1.2	0.36	0.894

TLC: total leucocytic count, LYM: lymphocytes, GRAN: granulocytes, MID: Indicates the combined value of the other types of white blood cells not classified as lymphocytes or granulocytes, RBCs: red blood cell count, HG: hemoglobin, HCT: hematocrite value, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, ALT: alanine transaminase, AST: aspartate aminotransferase, A/G ratio: albumin/globulin ration, SE: standard error

increase in pulse rate compared to the control group. However, the rectal temperature in the external parasite-infected group ( $39.03 \pm 0.03^{\circ}\text{C}$ ) was significantly higher than in the control group ( $38.76 \pm 0.04^{\circ}\text{C}$ ) ( $p=0.0066$ ) (see Table 5). As for the biochemical parameters, significant decreases were observed in the estimated ALT and albumin levels ( $p<0.05$ ) as well as total protein ( $p<0.0001$ ) in the infected group compared to the control group. In the blood of infected foxes, there was a notable increase in granulocytes, lymphocyte count, and total leukocyte count compared to the healthy control group ( $p \leq 0.05$ ). Additionally, indicators of anemia such as red blood cell count, hemoglobin, and hematocrit levels were significantly lower in foxes infected with ectoparasites compared to the control foxes ( $p \leq 0.05$ ) (Table 5). These findings suggest that foxes infected with external parasites suffer from significant health risks.

To further investigate the health condition of examined foxes and the associated changes in relation to parasitic infection, various systematic clinical findings were tested. Clinical signs were classified according to different body systems,

including digestive, respiratory, cardiovascular, and nervous systems, as well as general status (Table 6). Clinical signs in foxes infected with external parasites were mostly confined to general condition abnormalities, including weakness 85.7% (6/7), pale mucous membrane 85.7% (6/7), and anorexia 57.1% (4/7). Disturbances in general conditions and GIT functions have been reported when foxes were infected by internal parasites ( $n=4$ ). Such abnormalities include anorexia (50%), diarrhea (75%), vomiting (25%), weakness (50%), recumbency (25%) and pale mucous membranes (50%). While, more severe sickness was reported in foxes infected simultaneously with both internal and external parasites ( $n=8$ ). In such foxes, clinical findings also included nervous (ataxia, 37.5%), respiratory (nasal discharge, 25%; cough, 12.5%), and heart abnormalities (tachycardia, 50%). Additionally, general conditions comprising weakness (75%), recumbency (50%), pale mucous membrane (62.5%), and GIT, including anorexia (62.5%) and diarrhea (62.5%) have been recorded (Table 6). These additional investigations revealed the substantial impact of parasitic infections on the health condition of examined red foxes.

**Table 5:** Effect of ectoparasites infection on some clinical and laboratory parameters on red foxes (Adult male animals).

Tested parameters (unit)	Control (n=7)		Externally infected (n=3)		p-value
	Mean	SE	Mean	SE	
Temperature (Celsius degree)	38.76	0.04	39.03	0.03	0.0066
Pulse rate (pulse/1 min)	95.2	2.43	99.7	3.71	0.31
Respiratory rate (breath/1 min)	24.2	0.41	27.3	0.67	0.0028
TLC (10 <sup>3</sup> cells/mm <sup>3</sup> )	9.8	1.56	14.1	4.16	0.0015
LYM (10 <sup>3</sup> cells/mm <sup>3</sup> )	4.2	0.67	4.3	1.28	0.536
MID (10 <sup>3</sup> cells/mm <sup>3</sup> )	1.2	0.22	1.1	0.49	0.993
GRAN (10 <sup>3</sup> cells/mm <sup>3</sup> )	4.4	0.73	8.7	2.54	< 0.0001
LYM%	43.1	6.91	30.7	9.10	0.006
MID%	12	2.18	7.6	3.11	0.153
GRAN%	44.8	7.11	61.7	17.84	0.0004
RBCs (10 <sup>6</sup> cells/mm <sup>3</sup> )	12.4	1.94	10.4	3.00	0.0042
HG (g/dl)	16.2	2.53	12.2	3.54	0.0002
HCT (%)	47	7.36	37.8	10.93	0.0014
MCV (fl)	38	6.01	36.4	10.52	0.3841
MCH (pg)	13.1	2.06	11.8	3.40	0.0540
MCHC (%)	34.5	5.37	32.3	9.34	0.0711
Urea (mg/dl)	88.8	16.66	123.3	41.97	0.1791
Creatinine (mg/dl)	0.8	0.15	1.51	0.7	0.0759
ALT (U/l)	124.8	37.93	276	115.94	0.0283
AST (U/l)	99.2	26.24	142.3	61.35	0.2377
Glucose (mg/dl)	128.8	21.49	112	38.92	0.2310
Total proteins (g/dl)	8.8	1.36	6	1.95	< 0.0001
Albumin (g/dl)	5.4	0.84	3.7	1.08	0.0002
A/G ratio (numerical)	1.4	0.24	1.3	0.40	0.6621

TLC: total leucocytic count, LYM: lymphocytes, GRAN: granulocytes, MID: Indicates the combined value of the other types of white blood cells not classified as lymphocytes or granulocytes, RBCs: red blood cell count, HG: hemoglobin, HCT: hematocrite value, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, ALT: alanine transaminase, AST: aspartate aminotransferase, A/G ratio: albumin/globulin ration, SE: standard error

**Table 6:** Systematic clinical signs in parasitically infested (external, internal, and mixed infested) red foxes (*Vulpes vulpes*).

Affected system	Clinical sign	External (n = 7)	Internal (n = 4)	Mixed (n = 8)	Total (n = 19)
General status	Weakness	6 (85.7 %)	2 (50%)	6 (75%)	14 (73.7%)
	Recumbency	-	1 (25%)	4 (50%)	5 (26.3%)
Intestinal tract	Pale membrane	6 (85.7 %)	2 (50%)	5 (62.5%)	13 (68.4%)
	Anorexia	4 (57.1%)	2 (50%)	5 (62.5%)	11 (57.9%)
	Diarrhea	-	3 (75 %)	5 (62.5%)	8 (42.1%)
	Vomiting	-	1 (25%)	-	1 (5.3%)
Nervous system	Ataxia	-	-	3 (37.5 %)	3 (15.8 %)
Heart	Tachycardia	-	2 (50%)	4 (50%)	6 (31.6%)
Respiratory system	Nasal discharge	-	-	2 (25%)	2 (10.5%)
	Cough	-	-	1 (12.5%)	1 (5.3%)

(-) refers to not detected clinical signs for each category of parasite infection

## Discussion

Out of the 44 examined red foxes, 12 (27.3%) were found to be infected with at least one species of endoparasite either in postmortem examination or in collected fecal samples. The prevalence of nematodes was 27.3%, with two species identified. *T. canis* was the most prevalent at 27.3%, in line with the results obtained by Magi et al. (2016) in Italy (prevalence 26.7%) and significantly higher than Magi et al. (2009) in Italy (9.1%) and Miterpáková et al. (2009) in Slovakia (12.5%). However, this percentage is much lower than the figures reported by Suchentrunk and Sattmann, 1994

in Austria (42.9%) and Fiocchi et al. (2016) in Italy (52.5%). *Toxascaris leoninae* was less frequent than *T. canis* at 18.2%, which is significantly lower than the prevalence reported by Miterpáková et al. (2009) in Slovakia (42.9%) and Vervaeke et al. (2005) in Belgium (47.9%), but higher than figures reported by Dybing (2010) in Australia (4.7%) and Magi et al. (2009) in Italy (5.4%). Trematodes were only recorded in one female adult fox at a prevalence of 2.3%, which was similar to the results of Vervaeke et al. (2005) in Belgium (0.9%), Magi et al. (2016) in Italy (1.1%), and Dybing (2010) in Australia (1.4%).

The prevalence of ectoparasites identified (5



species) was 34.1%, which was similar to the findings of [El-Damarany \(1997\)](#) in Egypt. They identified six species, a much higher number than [Razmjo et al. \(2013\)](#) in Iran, who identified three species: *Ctenocephalides canis*, *Rhipicephalus* spp., and *Otodectes cynotis*. Additionally, the prevalence was lower than that found by [Dominguez \(2004\)](#) in Spain, who identified four species: *Ixodes Ricinus*, *Ixodes hexagonus*, *Haemaphysalis punctate*, and *Dermacentor reticulums*, and it was also less than the prevalence reported by [Millán et al. \(2007\)](#) in Spain, who identified six species: *Rhipicephalus pusillus*, *Rhipicephalus turanicus*, *Rhipicephalus* spp., *Ixodes Ricinus*, *Ixodes ventralloi*, and *Pulex irritans*.

The study found that the prevalence of fleas among red foxes was 34.1%. Four species of fleas were identified, with *Ct. canis* being the most prevalent at 25%. This rate was higher than the 12.9% recorded in a study in Iran but lower than the 53.8% found in Spain. *Ctenocephalidis felis* had a prevalence of 18.2%, similar to the 15.3% recorded in Spain and higher than the 1-2% reported in Austria. *Pulex irritans* had a prevalence of 4.5%, higher than the 0.58% found in Spain but lower than the 40% recorded in grey foxes from Mexico. *Echidnophaga gallinacean* had the lowest prevalence at 2.3%, a unique result for red foxes. The prevalence of ticks was 13.6%, with *Rippecephalus sangumatus* being the only species identified. This rate was higher than the 6.4% in Iran and 0.03% in Spain. Overall, the prevalence of both endo- and ectoparasites was highest in foxes from the Giza governorate (53.8%), followed by those from the Qena governorate (50%). Foxes from the Sohag governorate had the lowest prevalence rate at 36%. The study also noted variations in infestation rates among different geographical regions within the same country, which is a well-documented phenomenon. In this study, we looked into how internal or external parasite infections affect clinical, hematological, and biochemical parameters in infected and apparently healthy red foxes. We found that the infested group with internal parasites had significantly higher rectal temperature and respiratory rate compared to the control group. This aligns with the findings of previous reports on dogs from Egypt ([Salem and Farag., 2014](#)) and India ([Gonde et al., 2017](#); [Bilwal et al., 2017](#); [Panda et al., 2017](#)). However, we did not observe

these changes in foxes infected with internal parasites, which might indicate variations in animals' susceptibility to parasitic load.

The clinical examination results showed that pale mucous membranes and weakness were the most common clinical signs in the group externally infested with ticks. This was followed by enlarged lymph nodes and anorexia. However, there were no distinct clinical signs of involvement in the nervous, cardiovascular, or respiratory systems. These results were consistent with those obtained by [Bilwal et al. \(2017\)](#) in dogs from India, who found weakness to be the prominent clinical sign at 68%, followed by pale mucous membrane at 56% and anorexia at 50%. Similar results were reported by [Sarma et al. \(2015\)](#) in dogs infected with ticks from India, with 100% incidence of pale mucous membrane and enlarged lymph nodes, 86% weakness, and 77% anorexia. [Gonde et al. \(2017\)](#) also published similar results in dogs from India. The internally infested group showed a high incidence of diarrhea (75%) and the same incidence of weakness, pale mucous membranes, tachycardia, and anorexia (50% for each). There was less incidence of recumbency and vomiting (25% for each). This was consistent with the findings of [Osman et al. \(2015\)](#), who recorded an 84% incidence of anorexia, 12.7% pale mucous membrane, and 11.11% diarrhea. Similar findings were reported by [Lefkaditis et al. \(2006\)](#) in dogs from Romania. [Aref et al. \(2018\)](#) also recorded similar clinical signs, including emaciation, diarrhea, off-food, and pale mucous membrane in dogs from Egypt infected with *T. canis* and *T. leoninae*.

In the group infected by both internal and external parasites, weakness was recorded with an incidence of 75%, followed by pale mucous membranes, anorexia, and diarrhea (62.5% for each). Recumbency, enlargement of lymph nodes, and tachycardia were each recorded with a 50% incidence. Ataxia was a prominent nervous sign recorded at 37.5%, nasal secretions represented 25%, and the least incident clinical sign was cough (12.5% for each). These results were consistent with those from [Bilwal et al. \(2017\)](#) in dogs from India, who also recorded ataxia at 31.25%, nasal discharge at 18.75%, and coughing at 18.75%, in addition to weakness, pale mucosa, diarrhea, and others.

The recent study results showed that the total leukocytic count (TLC) in infected animals was significantly higher than in apparently healthy animals. These findings were consistent with studies by [Salem et al. \(2015\)](#) on *T. canis* infected dogs from Egypt, [Chattha et al. \(2009\)](#) on *T. canis* infected dogs from Pakistan, and [Kebbi et al. \(2020\)](#) on *Rhipicephalus* sp.-heavily infected dogs from Algeria. However, these results were not in line with the findings of [Aref et al. \(2018\)](#) on different intestinal nematode-infected dogs from Egypt, who observed an increase in TLC in infected dogs without statistical significance. [Ogunkoya et al. \(2006\)](#) also reported similar findings in intestinal helminth-infected dogs from Nigeria. The marked leukocytosis in infected foxes may be referred to as concurrent infection.

In terms of the cellular immune response, the granulocytic counts in infected animals were higher than those of apparently healthy animals, which was similar to results reported by [Kumar et al. \(2014\)](#) in *T. canis* infected dogs, [Bilwal et al. \(2017\)](#) in dogs from India, who recorded a significant increase in neutrophils in infected animals, and in the case of [Sarma et al. \(2015\)](#) in ticks harboring dogs from India, although the difference was not significant. Lymphocytic percentages in infected animals were lower than those of apparently healthy animals, and these results were in agreement with [Kumar et al. \(2014\)](#) in *T. canis* infected dogs from India, [Katariya et al. \(2018\)](#) in dogs from India harboring different external parasites, and [Bilwal et al. \(2017\)](#) in dogs from India. The granulocytic percentage in infected animals was significantly higher than that of apparently healthy animals, which was in accordance with [Bilwal et al. \(2017\)](#), [Selvaraj et al. \(2010\)](#), and [Shah et al. \(2011\)](#) in dogs from India. Total erythrocytic count (TEC), hemoglobin, and hematocrit values in externally parasitized animals were significantly lower than those of apparently healthy animals, and these results were in accordance with [Yogeshpriya et al. \(2018\)](#) and [Sarma et al. \(2015\)](#) in ticks harboring dogs from India. Reduction in erythrogram in parasitized foxes compared to control foxes, suggesting anemia.

Nevertheless, induction of serum hemolytic factors increased erythro-phagocytic activity of macrophages, and damage induced by the secondary immune system after the formation of

anti-erythrocyte membrane antibodies is also important in the pathogenesis of anemia. The results of the present study revealed that the foxes were anemic and dehydrated as compared to non-infested animals due to fleas and tick infestation. It is well known that anemia and inflammatory stimuli influence the release of thrombocytes from the spleen pool or the bone marrow. There were no significant differences in percentages of MCV, MCH, and MCHC among apparently healthy animals and infected animals with internal or external parasites during this study.

In this study, the ALT levels in infected animals were found to be significantly higher than those of apparently healthy animals. These results are consistent with previous studies: [Atasoy et al. \(2015\)](#) observed similar findings in dogs from Turkey infected by *T. canis* and *T. leoninae*, while [Kumar et al. \(2014\)](#) found the same in *T. canis* infected dogs from India, and [Yogeshpriya et al. \(2018\)](#) observed elevated ALT levels in ticks harboring dogs from India. The excessive release of the enzyme may be linked to increased permeability of liver cells of ALT enzyme into the bloodstream, leading to elevated levels in the serum. This could be interpreted as the effects of *T. canis* infection on the liver ([Atasoy et al., 2015](#)). In the current study, the obtained results revealed that glucose levels in infected animals were significantly lower than those of apparently healthy animals. These results were consistent with [Atasoy et al. \(2015\)](#) in dogs from Turkey infected by *T. canis* and *T. leonine*, [Katariya et al. \(2018\)](#) in dogs from India harboring different external parasites, and [Solanki and Hasnani \(2006\)](#) in dogs from India suffering from demodicosis. However, non-significant differences were obtained in the case of externally parasitized foxes.

Total serum protein and albumin in infected animals were significantly lower than those of apparently healthy, and this result matched with [Atasoy et al. \(2015\)](#) in dogs from Turkey infected by *T. canis* and *T. leoninae*, [Sarma et al. \(2015\)](#) in ticks harboring dogs from India, [Kumar et al. \(2014\)](#) in *T. canis* infected dogs from India, while disagreeing with [Yogeshpriya et al. \(2018\)](#) in ticks harboring dogs from India, who found no significant differences neither in serum total protein nor albumin among parasitized and non-parasitized dogs. The marked hypoproteinemia may be due to chronic internal hemorrhage

during infection and loss of serum through exudation or leakage in the lumen of the gut, causing enteropathy. Hypoproteinemia may also be attributed to the interference with the efficacy of digestion and absorption by damaged intestinal mucosa and diarrhea due to mechanical irritation (Kumar et al., 2014). Hypoproteinemia and hypoalbuminemia might be due to a chronic inflammatory disease, anorexia, or decreased protein intake (Mylonakis et al., 2010). There were no significant differences in values of urea, creatinine, and albumin/globulin ratio among adult-infected and non-infected foxes. As albumin is one of the smallest of the plasma proteins, it tends to be lost more readily than the others, and so these conditions often present primarily as hypoalbuminemia (Kerr, 2008). Hypoproteinemia could be attributed to the interference with the efficacy of digestion and absorption by intestinal mucosa due to nematode infection (Kumar et al., 2014).

## Conclusion

The red fox (*Vulpes vulpes*) is the most widely distributed and adaptable carnivore in the world, including Egypt, either in rural or urbanized regions. Herein, 44 red foxes from different ages, sexes, localities of Egypt (Giza, Sohag, and Qena governorates), and periods were involved in this study. Our data demonstrated the high existence of *T. canis* (27.3%), *T. leoninae* (18.2%), and Trematode spp. (4.5%). Also, various species of fleas as *Ct. canis* (25%), *Ct. felis* (18.2%), *P. irritans* (6.8%), and *E. gallinacea* (4.5%), and one tick species, *R. sanguineus* (13.6%) were recorded in examined foxes. Numerous clinical and biochemical variables were examined to assess the effect of parasitic infection in red foxes. A marked decrease in total protein and albumin levels in infected foxes than the non-infected group was the paramount finding, indicating nutritional disturbances. This study suggests red fox is a pivotal player in various Egyptian ecological systems. This effect is extrapolated from the high existence of various internal and external parasites that would be detrimental not only for foxes, as confirmed here, but also for other animals and humans.

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