



## Epidemiological and Diagnostic study of *Toxoplasma gondii* in Cats and Humans Using Microscopic and Serological Methods in Shahat City

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**Abstract**—*Toxoplasma gondii*, the causative agent of toxoplasmosis, represents a significant public health concern due to its wide distribution and zoonotic potential between cats and humans. This study aimed to investigate the occurrence of *T. gondii* in cat feces and human blood within the same study area (Shahat City). Cat fecal samples examined using Sheather's Sugar Flotation Technique, and human blood samples analyzed using the enzyme-linked immunosorbent assay (ELISA) technique. The overall prevalence of *T. gondii* infection in Shahat City was 65%. The infection rate among female cats (84.6%) was significantly higher than that of male cats (15.3%). Regarding lifestyle, free-roaming cats showed the highest prevalence (41.0%), followed by semi-confined cats (38.4%), and while indoor confined cats had the lowest rate (20.5%). By age, cats older than two years up to more than four years exhibited the highest infection rate (46.1%), whereas the lowest rate was in the 12–24 month age group (20.5%). Seasonally, prevalence was highest in summer (80.0%), followed by spring (69.3%), autumn (54.6%), and lowest in winter (23.0%). Furthermore, serological analysis of human blood samples demonstrated that 32.2% of individuals were positive for IgM, while 78.9% were positive for IgG. These findings highlight the urgent need for strategies to minimize and control the prevalence of toxoplasmosis.

**Keywords**— Toxoplasmosis; *Toxoplasma gondii*; IgM; IgG; Shahat City

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### 1. Introduction

Parasitic diseases represent some of the most complex global health challenges, posing a persistent threat due to their wide distribution and direct impact on both humans and animals. Recent studies indicate that protozoan parasites are responsible for substantial number of chronic infections in both developing and developed countries, making them a central focus of research in public health and veterinary medicine [1]. Among these diseases, toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii*), is considered one of the most significant zoonotic infections worldwide. It is estimated that nearly one-third of the global population is at risk of toxoplasmosis, a reflection of the parasite's remarkable adaptability and its ability to infect a wide range of animal hosts [2].

*T. gondii* is an obligate intracellular parasite, and cats serve as the only definitive hosts capable of shedding oocysts in their feces. Other mammals, including humans, act as intermediate hosts that sustain the parasite's transmission cycle in the environment. Human infection typically occurs through ingestion of food or water contaminated with oocysts from cat feces, direct hand-to-mouth contact, or consumption of raw or undercooked meat from infected animals such as sheep and birds [3]. Toxoplasmosis poses a particular public health concern for pregnant women due to the potential for vertical transmission through the placenta, which can result in severe complications including spontaneous abortion, congenital malformations, or fetal death [2,4,5]. According to a joint report by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), toxoplasmosis ranks among the four most serious foodborne diseases worldwide, largely due to its strong association with food-handling practices and exposure to infected animals [6].

Recent epidemiological analyses suggest that the prevalence of *T. gondii* is closely associated with the level of public health awareness with local communities. Accurate diagnosis is essential for effective disease control. Serological assays, particularly the Enzyme-Linked Immunosorbent Assay (ELISA), remain the most sensitive and reliable tools for detecting specific IgM and IgG antibodies against the parasite [4]. Moreover, recent studies highlight the importance of integrating advanced molecular techniques with traditional serological approaches to enhance diagnostic accuracy, especially in complex epidemiological contexts [7]. With rapid urbanization and increased interaction between humans and domestic animals, exposure to *T. gondii* has risen in many regions, particularly those with high in areas with high population density and warm, humid climates that favor oocyst survival and transmission [4].

From an epidemiological standpoint, monitoring the spread of *T. gondii* infection in humans provides a valuable indicator of overall public health status, as infection patterns in humans and cats often mirror each other due to their close ecological relationship within shared environments [2]. In Libya, however, the absence of comprehensive epidemiological studies has created a gap in understanding the true burden and transmission dynamics of toxoplasmosis. Given the scarcity of data on *T. gondii* in the country, the present study aims to address this gap by determining the prevalence of *T. gondii* oocysts in cats and assessing IgM and IgG antibodies in humans residing in Shahat City. By examining the epidemiological relationship between feline infection and human toxoplasmosis within the same area, this study contributes to a deeper scientific understanding of the disease and supports the development of effective prevention and control strategies.

## **2. Materials and Methods**

### **2.1. Description the area of study**

Shahat City, one of the most famous ancient cities in Libya, is located in north-west Libya at approximately 32.822 N° and 21.86 E°. The area situated 12 km from the Mediterranean coast, at an elevation of about 620 meters above sea level.

### **2.2 Feces collection and analysis**

Studied samples were collected from January to December 2024. A total of 300 African cat fecal samples were obtained from various environmental sources, including public streets, squares, and residential backyards in Shahat City, Libya, at a rate of 75 samples per season. Each sample was placed in properly labeled plastic container and preserved in isothermal containers at 4°C until

processing in the laboratory. The study was conducted on the African cat breed, and data on the animals were recorded, including age, sex, lifestyle, and feeding habits. Sheather sugar flotation technique was used to identify *T. gondii* in cat feces microscopically; the technique was performed as described by Sheather [8].

### 2.3. IgM and IgG detections

Human antibodies tests, IgM and IgG, were carried out on a total of 220 females, and blood samples were obtained from women of different age groups. A 5 ml blood sample was collected from each participated female. The blood samples were taking in vials containing an anticoagulant (EDTA), and centrifuged at 3000 rpm for 10 minutes. To detect the presence of antibodies (IgM and IgG) the serum was separated and tested using the ELISA test. The results were recorded according to the manufacturer's instructions [8].

### 2.4. Statistical analysis

The study utilized the chi-square test for two or more variables. Other statistical analyses were conducted using the Minitab 2014 software, with an Analysis of Variance (ANOVA) test applied where a 95% confidence level was adopted.

## 3. Results

Using Sheather's microscope examination technique, the study found that 195 (65%) of African domestic cat fecal samples were contaminated with *T. gondii* oocysts, whereas 105 (35%) were non-infected (Table 1).

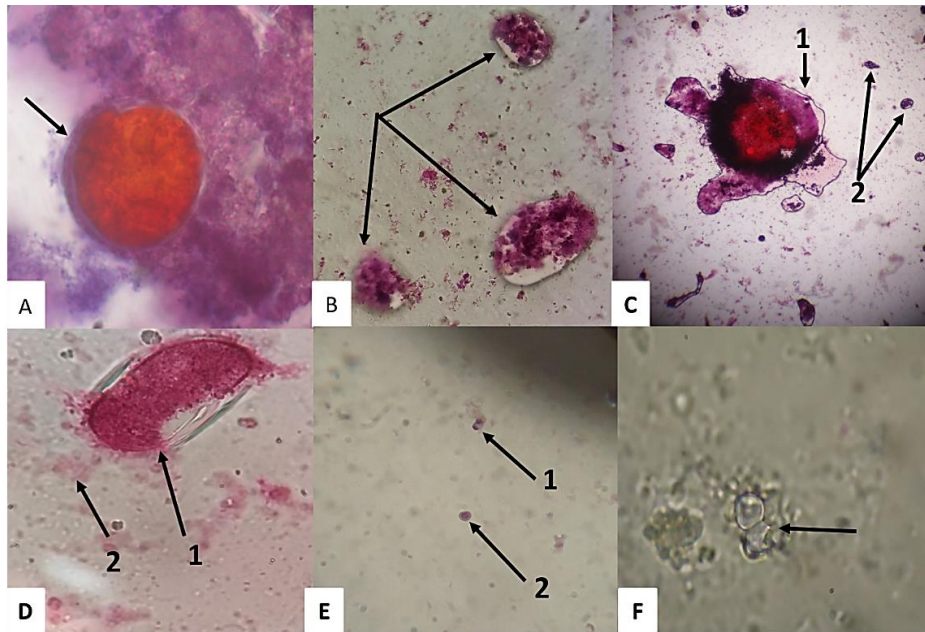
**Table 1.** The percentage of toxoplasmosis infection among African domestic cats in Shahat City

The presence of oocysts in fecal samples	Frequencies	Percentage
Positive samples	195	65%
Negative samples	105	35%

Microscopic examination of tested feces revealed the presence of *T. gondii* pre-oocysts in all samples, measuring 6-9  $\mu\text{m}$  in length and 7-8  $\mu\text{m}$  in width (Fig. 1 A and C). These pre-oocysts are considerably smaller than the mature *Toxoplasma* oocysts (Fig. 1 B and C), which appear red in color and measure 8-11  $\mu\text{m}$  in length and 7-11  $\mu\text{m}$  in width. The tachyzoite stage was also observed during examination, as illustrated in Fig. 1 D. Additionally, some infected samples in Fig. 1 (E and F) showed spherical or ovoid forms (sporulated & unsporulated) with a diameter ranging from 10-12  $\mu\text{m}$ .

Data in Table 2 showed various significant differences between infected and non-infected cats according to sex, lifestyle, age and season. Toxoplasmosis prevalence among females was much higher than males ( $P = 0.014$ ). Regarding lifestyle habits, free-ranging cats recorded the highest infection rate (41.0%), followed by semi-confined cats (38.4%), while confined indoor cats had the lowest prevalence (20.5%), with a statistically significant association between lifestyle and infection rate ( $P = 0.016$ ). In terms of age groups, cats aged 24 months to more than 4 years had the highest infection rate (46.1%), whereas the lowest prevalence was recorded in 12-24 month age group (20.5%), with a highly significant difference between age and infection rate ( $P = 0.002$ ).

When the infection rates were analyzed by season, the highest prevalence was observed in summer (80.0%), followed by spring (69.3%), autumn (54.6%), and winter (23.0%). The difference between seasons was statistically insignificant ( $P = 0.07$ ).



**Figure 1.** Microscopic examination of fecal samples from an infected cat demonstrating the different developmental stages of *T. gondii*: A- Pri-oocysts, B- Mature oocysts, C- The black arrows showed: (1) pri-oocysts (2) mature oocysts, D- The black arrows showed: (1) oocyte filled with tachyzoites, (2) free tachyzoites, E- The black arrows showed (1) un-sporulated oocysts, (2) sporulated oocysts. F- Un-sporulated oocysts.

**Table 2.** The percentage of toxoplasmosis infection in tested cats according to sex, lifestyle, age and season

Variables	P-value	Sub-variables	% of infected cats	% of non-infected cats
Sex	0.014	Male	15.3%	66.6%
		Female	84.6%	33.3%
Life style	0.016	Confined	20.5%	57.1%
		Semi-confined	38.4%	29.5%
		Free-ranging	41.0%	13.3%
Age	0.002	1 – 12 months	33.3%	28.5%
		12 – 24 months	20.5%	42.8%
		24 – 48 months	46.1%	28.5%
Season	0.070	Spring	69.4%	30.6%
		Summer	80.0%	20.0%
		Autumn	54.6%	45.4%
		Winter	23.7%	69.3%

Regarding serological examinations, a total of 220 women were tested using the ELISA technique to detect the presence of *Toxoplasma* antibodies, specifically IgM and IgG. The results showed that 32.2% of the tested female samples were positive for IgM antibodies, while 68.0% of them were negative. The examination also revealed that 78.9% of the women were positive for IgG antibodies, while the remaining 21.0% were negative. Based on these findings, the prevalence of IgG was higher among infected females in the Shahat area (Table 3).

**Table 3.** Percentage of IgM & IgG antibodies in female blood

Antibody Type	Number of Samples(n)	Test Result	Percentage (%)
IgM	40	Positive	32.0
IgM	85	Negative	68.0
IgG	75	Positive	78.9
IgG	20	Negative	21.0

#### 4. Discussion

The presence of *T. gondii* oocysts in cat feces is the primary mechanism through which the parasite is dispersed into the environment. A higher burden of oocysts shed by cats directly increases the environmental load of infective stages, thereby elevating the risk of exposure and, consequently, the prevalence of toxoplasmosis in susceptible populations. This study represents the first of its kind in Shahat City, Libya, as it combines fecal examination to determine the prevalence of the toxoplasmosis in cats with the detection of Toxo IgM and IgG antibodies in humans using the ELISA technique within the same region.

Toxoplasmosis infection among African cats in Shahat City varied according to season, age, lifestyle, and sex. In addition, the presence of infected cats in close proximity to humans in the same area increased infection rates and contributed to serious health problems, particularly among pregnant women. These findings confirm that cats are a major source of toxoplasmosis infection and transmission in the study area. The current results are consistent with several recently published studies [9,10,11]. Shahat City appears to provide favorable environmental and ecological conditions for the persistence and spread of *T. gondii*.

The study revealed a *T. gondii* oocysts prevalence of 65% in cats in Shahat City. This percentage differs markedly from recent studies conducted in Al-Beida (29.4%) and Al-Marj (11%), located about 15 km and 130 km west of Shahat, respectively. This discrepancy may be attributed to several factors, including differences in cat-rearing practices, availability of veterinary healthcare, and variations in household hygiene habits related to preventive measures when handling cats. The lower prevalence reported in Al-Marj may be due to, at least in part, to the small sample size analyzed.

Regarding the immunological examinations, most of the women tested were IgM-negative (68%) and IgG-positive (78.9%), indicating that the majority had past infections. In contrast, a smaller proportion of women were IgM-positive (32%) and IgG-negative (21.1%), suggesting that a limited number of participants had recent or acute infections. These findings highlight the endemic nature of toxoplasmosis in the region and reflect ongoing transmission of *T. gondii* to humans. Such a situation represents a particular threat to pregnant women and immunocompromised individuals. The results align with a previous study [8], which found higher rates of past infection compared to recent infection among pregnant women in Al-Beida City, Libya. Collectively, these findings indicate ongoing transmission in both regions and an increased public health risk.

Overall, the results reinforce the understanding of toxoplasmosis as a zoonotic parasitic disease requiring integrated public health monitoring. It represents a silent threat, particularly pregnant women due to the risk of congenital malformations, spontaneous abortion, or neonatal death, in addition to immunocompromised individuals. Reducing indoor cat ownership and limiting direct contact with cats are recommended, as they represent a primary source of *T. gondii* infection.

Enhancing public health awareness and implementing routine monitoring programs by relevant authorities are essential to reduce transmission risk.

## 5. Conclusion

This study highlights the high prevalence of *T. gondii* infection in Shahat City, emphasizing its significant zoonotic risk to public health. The observed infection patterns, shaped by factors such as sex, lifestyle, age and season, provide valuable epidemiological insights into the dynamics of parasite transmission between hosts. Serological detection of both IgM and IgG antibodies in humans indicates the presence of active and past infections, reflecting continuous exposure within the community. These findings underscore the urgent need to enhanced public awareness, improved hygiene practices, and implement effective preventive and control strategies to limit the spread of toxoplasmosis and reduce its impact on public health.

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