

Seroprevalence and Risk Factors of Toxoplasmosis in Domestic Cats Brought to Small Animal Clinics in Kampala District, Uganda

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Abstract

Cats, as the definitive hosts of *Toxoplasma gondii*, play a critical role in the transmission of this ubiquitous zoonotic protozoon parasite. Despite the public health importance of *T. gondii* and the central role of domestic cats in its epidemiology, there is a notable lack of data on its prevalence in cats in Uganda, and no studies have specifically investigated this issue to date. To address this knowledge gap, we sampled blood from 80 cats brought to selected small animal clinics in Kampala District Central Uganda, and determined their *T. gondii* seroprevalence using an enzyme linked immunosorbent assay (ELISA) antibody test. Multivariable logistic regression analysis was performed to determine the sociodemographic factors associated with *T. gondii* seropositivity. The seroprevalence was 45.2% (95% CI: 41.1 - 62.5). The analysis revealed several factors positively associated with Toxoplasmosis seropositivity ($p \leq 0.25$) among domestic cats. These included: raw food consumption (aOR 6.76, 95% CI: 1.38 - 33.12, 0.018), roaming outdoors (aOR 6.20, 95% CI: 1.54 - 24.95, 0.010) and adult/senior age group (aOR 3.82, 95% CI: 1.20 - 12.20, 0.023). These findings underscore the need for enhanced surveillance, routine screening, improved public awareness, and targeted preventive measures to reduce the burden of toxoplasmosis in Kampala District, Uganda, and the wider East African region.

Keywords

Cats, Toxoplasma Gondii, Seroprevalence, Risk Factors, Antibodies

1. Introduction

Toxoplasmosis is a ubiquitous zoonotic disease caused by the obligate intracellular protozoan parasite *Toxoplasma gondii* and represents a major public health concern worldwide [1]. The parasite is capable of infecting virtually all warm-blooded animals, especially livestock and humans [2] [3]. *T. gondii* exists in three main forms: oocysts, which are shed by the definitive feline host into the environment; and bradyzoites and tachyzoites, which are found within the tissues of intermediate hosts [4]. Livestock are particularly susceptible to infection when reared on pasture, where they may ingest environmentally resistant oocysts contaminating soil, water, or forage [4]. Humans acquire the infection through various routes, from the consumption of undercooked meat, contact with contaminated soil or water, and, most notably, contact with infected cats and their faeces [5]. Although domestic cats are the only definitive hosts responsible for shedding oocysts into the environment, the extent to which direct contact with cats contributes to human toxoplasmosis remains an ongoing subject [6].

Domestic cats (*Felis catus*) play a crucial role in the epidemiology of toxoplasmosis as the definitive host [7]. Following ingestion of infected prey, cats become infected and shed oocysts in their faeces for approximately 1 - 3 weeks [8] [9]. These environmentally resistant oocysts can remain viable for prolonged periods, surviving for up to 1.5 years in moist soil and 4.5 years in cool freshwater [10]. Although oocyst shedding is typically transient, re-shedding may occur without re-ingestion of tissue cysts when cats experience concurrent intestinal infections [11]. The intermediate hosts can then acquire the infection by ingesting oocysts from contaminated soil, food, and water [12]. After ingestion, oocysts release sporozoites that differentiate into tachyzoites, which invade host cells, multiply rapidly, and disseminate throughout the body, causing tissue damage [12]. In some cases, the infection becomes chronic, with the formation of tissue cysts containing bradyzoites [4].

Early detection of *T. gondii* infection in cats, followed by appropriate treatment with clindamycin, pyrimethamine, and/or sulphonamides, together with improved hygiene and confinement, is essential for reducing environmental contamination and minimizing the risk of transmission to humans and other animals. Diagnosing toxoplasmosis in cats typically involves a three-pronged approach: considering the cat's history, clinical signs, and laboratory tests. Faecal examination for oocysts is a relatively simple and direct approach but it is mainly limited by the brief shedding period [8] [9]. Consequently, serology is the most commonly utilized approach for identifying cats exposed to *T. gondii* by detecting *T. gondii*-specific Immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies in the serum [13]. Common serological techniques used for Toxoplasmosis diagnosis include latex agglutination test (LAT), indirect hemagglutination assay (IHA), western blotting and enzyme-linked immunosorbent assays (ELISA) [5] [14]-[16].

Domestic cats are known to be the primary host for this ubiquitous intracellular parasite. In Uganda, previous studies have primarily investigated the seroprevalence of *T. gondii* in intermediate hosts, including chickens (47%), goats (31%)

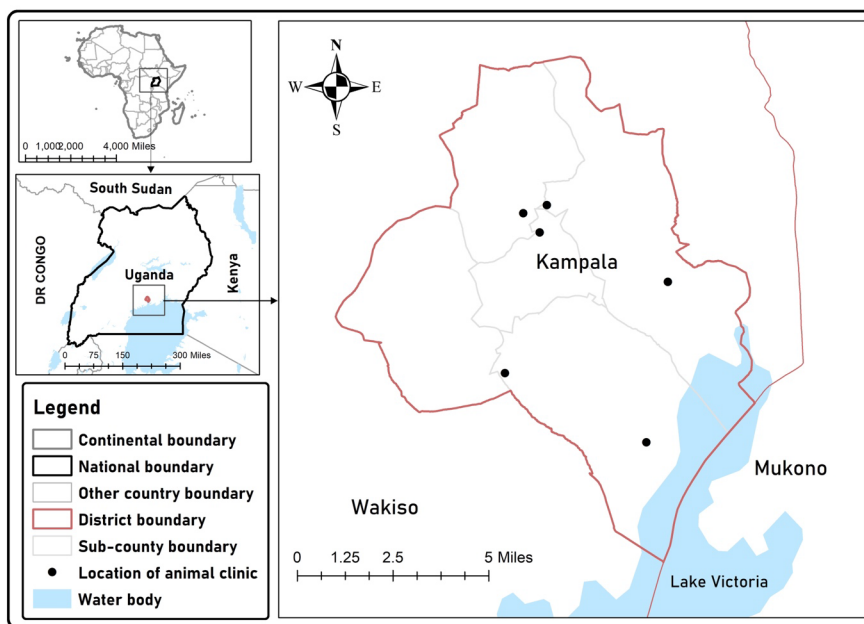
[17], pigs (28.7%) [18] and humans, where a seroprevalence of 54% has been reported in HIV positive patients [19]. However, there is a paucity of data on the seroprevalence of *T. gondii* infection in cats, despite their pivotal role in the parasite's life cycle and transmission.

This knowledge gap limits understanding of the epidemiology of toxoplasmosis and hinders accurate assessment of the risk of environmental contamination and transmission to humans and other animals. Generating epidemiological data from the definitive host is crucial for informing evidence-based surveillance and developing effective prevention and control strategies. Therefore, this study aimed to determine the seroprevalence of *T. gondii* infection in domestic cats in Kampala District, Uganda, and to identify potential risk factors associated with seropositivity.

2. Materials and Methods

2.1. Study Area

Kampala, the capital of Uganda, is the country's largest urban centre and one of the fastest-growing cities in Africa, with an annual population growth of approximately 5.6% [20]. Pet ownership is common amongst city residents. Cats and dogs are the most commonly kept companion animals [21]. According to the Uganda Bureau of Statistics, 2021, the estimated population of owned domestic cats in Kampala District is 9,054. The increasing pet population has been accompanied by a corresponding growth in the number of small animal veterinary clinics that provide healthcare services to companion animals.



Note: This map was created by Author Dr. Lewis Ashabahebwa.

Figure 1. Map of Kampala district showing the location of small animal clinic (round dots).

This study was conducted in six small animal veterinary clinics located across

four administrative divisions of Kampala District: Central, Kawempe, Makindye, and Nakawa. The geographical distribution of the participating clinics is shown in **Figure 1**.

2.2. Study Population and Sample Size

Pet cats in Uganda are commonly allowed to roam freely [22] [23], hence recruiting animals directly within a home setting was considered logistically challenging. Therefore, the study population comprised of cats presented to small animal clinics within Kampala District. Clinics were selected based on their registration status with the regulatory authority and the average number of feline patients received per month. Clinics that were not registered with the Uganda Veterinary Board (now the Uganda Veterinary Council [UVC]) and/or received fewer than two feline patients per month were excluded. A list containing eight veterinary clinics within Kampala District was obtained from the UVC. Each clinic was contacted by telephone to assess its eligibility and invite participation in the study. Of the eight clinics contacted, six clinics met the inclusion criteria and consented to participate in this study.

To determine the sample size, a preliminary survey was conducted to estimate the total number of cats brought to the selected small animal veterinary clinics over a three-month period. The survey estimated that approximately 100 cats would be presented during this period. The required sample size was then calculated using the Krejcie and Morgan formula for finite populations [24]:

$$S = X^2 NP(1 - P) \div d^2 (N - 1) + X^2 P(1 - P)$$

X^2 = table value of chi-squared for 1 degree of freedom at the desired CI (3.841);

N = the population size (100);

P = population proportion (assumed to be 0.50) since this would provide maximum sample size;

d = the degree of accuracy expressed as a proportion (0.05).

Therefore, $S = 80$ cats.

2.3. Sample Collection

Sample collection was conducted between July and September 2025. All cats presented to the selected small animal veterinary clinics during the study period were eligible for inclusion, except cats younger than 3 months of age, which were excluded because maternally derived antibodies may interfere with serological testing. Failure to obtain a blood sample from the respective cat led to its exclusion from the study. Written informed consent was obtained from all participating cat owners before sample collection. Owners completed a structured questionnaire to collect information on cat and owner-related characteristics for assessment of potential risk factors associated with *T. gondii* infection.

A total of 106 cats met the inclusion criteria. Of these, owners of 95 cats consented to participate in the study. Blood samples were successfully collected from

83 cats; however, three samples yielded insufficient serum for serological analysis. Consequently, serum samples from 80 cats were included in the final analysis. Blood samples were aseptically collected from cats via venipuncture of the cephalic vein using sterile needles and heparinized vacutainer tubes. The venipuncture site was properly occluded and disinfected prior to sampling to ensure aseptic collection. All blood collections were performed by one of two trained veterinary professionals on the research team.

Immediately after collection, the blood samples were placed in a cooler box containing ice packs to maintain cold chain during transport to the laboratory. Upon arrival, the samples were centrifuged to separate the serum, which was aliquoted into sterile, labelled cryovials. A total of 80 serum samples were stored at 2°C - 8°C for short-term preservation and subsequently at -20°C until serological analysis for *Toxoplasma gondii*.

2.4. Serological Assay

Serum samples were analyzed for antibodies against *Toxoplasma gondii* using a commercial indirect enzyme-linked immunosorbent assay (ELISA) kit (Feline *Toxoplasma gondii* Antibody ELISA Kit; Ring Biotechnology Co., Ltd., Product No. PZ5024), following the manufacturers instructions. Serum samples were first diluted with the buffer at a ratio of 1:200 µL. Each sample was run in duplicates to obtain a mean optical density (OD). A positive control (PC) and negative control (NC) were also run to ensure the validity of the test results.

After sample dilution, the microplate was covered and incubated at 37°C for 30 minutes then washed three times with the wash buffer. The enzyme conjugate was then added and the plate incubated at 37°C for 30 minutes. Following a second washing step, tetramethylbenzidine (TMB) substrate was added, and the plate covered and incubated again at 37°C for 10 minutes. The reaction was stopped using the stop solution and ELISA plate was read immediately at 450 nm using an ELISA microplate reader.

Assay validity was determined according to the manufacturer's acceptance criteria. The mean OD value of the positive control (PC) was required to be ≥ 0.50 , while that of the negative control (NC) was required to be ≤ 0.20 . Sample results were interpreted using the following formula:

$$S/P = (\text{Mean OD of sample} - \text{NC}) / (\text{PC} - \text{NC})$$

For a sample to be positive, the S/P greater than or equal to 0.2;

For a sample to be negative, the S/P < 0.2.

2.5. Data Collection

1. Assessment of Risk Factors

Data on potential risk factors associated with *T. gondii* infection were collected using a researcher-administered questionnaire comprising 10 closed-ended (multiple-choice) questions and 12 open-ended questions. cat demographic characteristics (age, sex, and neuter status), housing conditions (type of environment, pres-

ence of other animals or cats in the household, and interaction with other cats), outdoor access, diet, source of the cat (rescued from the street, adopted, or purchased from a breeder), and availability of a litter box. Information on owner-related characteristics was also collected to facilitate assessment of factors potentially associated with *T. gondii* seropositivity.

2. Ethical Approval

All procedures and protocols used in this study were approved by the School of Veterinary Medicine and Animal Resources Institutional Animal Care and Use Committee (SVAR-IACUC/212/2024). Participation was voluntary, and written consent was obtained from cat owners. For owners below the age of 18, written consent was obtained from a parent or legal guardian.

2.6. Data Analysis

Data were analysed using STATA Version 15.0. Descriptive statistics were used to summarize the study variables. Frequencies and percentages were used to report categorical variables such as cat sex, housing type, sterilization status, and feeding practices. Mean and standard deviation (SD) were used to present continuous variables such as the owner's age. Median and interquartile ranges were reported for data that were not normally distributed.

The seroprevalence of Toxoplasmosis was calculated as the percentage of cats that tested positive with the ELISA test. The true prevalence and its corresponding confidence intervals were estimated after adjusting for the diagnostic test's sensitivity (87%) and specificity (75%), according to the manufacturer instruction, using the following formula:

$$\text{True prevalence} = \frac{(\text{Apparent prevalence} + Sp - 1)}{(Se + Sp - 1)}$$

We performed a bivariate analysis using simple logistic regression to explore associations between independent variables and *T. gondii* seropositivity. Variables with a p-value of less than 0.25 were considered for inclusion in the multivariable analysis, as well as those of epidemiological significance or established theoretical relevance. Potential clustering of cats from the same clinics was considered to be minimal because sampled cats originated from different households with varying management practices; therefore, independence of observations was assumed.

Multicollinearity was assessed among the selected variable using Variance Inflation Factor with VIF > 10% as the threshold of exclusion, no variable met this criterion. For multivariable analysis, we employed a manual backward elimination logistic regression process rather than an automated algorithm. This prevented early removal of theoretically relevant variables, guided by statistical significance, and allowed us to check for confounding at each step. To assess the latter, we monitored changes in regression coefficients after removal of each variable using a threshold of >10% change to indicate potential confounding.

Adjusted Odds Ratios (aOR), their 95% confidence intervals and their p-values were reported to describe the magnitude and direction of the associations. The final model was significant (Omnibus test $\chi^2 = 37.468$, $p \leq 0.001$), and it fit the data well as indicated by the Hosmer-Lemeshow goodness of fit test ($p = 0.619$). The model's predictive power was validated by its correct classification of 74.7% of the observations.

3. Results

3.1. Background Characteristics of Cat Owners in Kampala District

The respondents presented their cats at 6 clinics; the minimum number registered at a clinic was 11 and the highest number was 27 over a period of 3 months. Majority of owners were male 54.2%. The mean age was 34.7 years, with 53.0% below the age of 30. Most owners (56.6%) had tertiary education, 26.5% had primary education, 12.0% had completed secondary school, and 4.8% had no formal education. A minor number lived in huts (7.2%).

3.2. Demographic Characteristics of the Cats Treated at Selected Animal Clinics in Kampala District

Majority of cats (39.2%) were below 2 years old and 53.7% were male (Figure 2). Most cats were rescued from the streets (58.7%) and more than half (52.5%) were neutered.

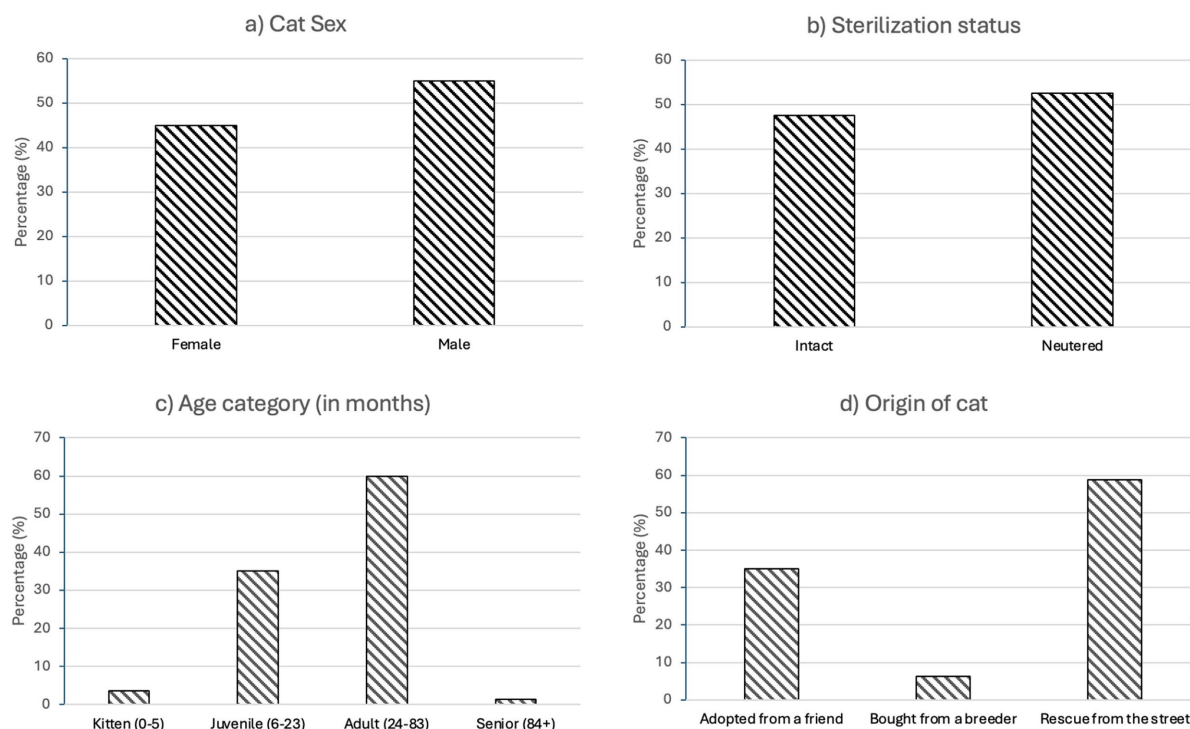


Figure 2. Demographic characteristics of cats brought to small animal clinics in Kampala District: sex, sterilization status, age and origin.

The household environment showed that 53% of the cats lived with other cats. Additionally, 66.3% of households had other animals. Outdoor access was common and majority of cats were fed processed food as seen in **Figure 3**.

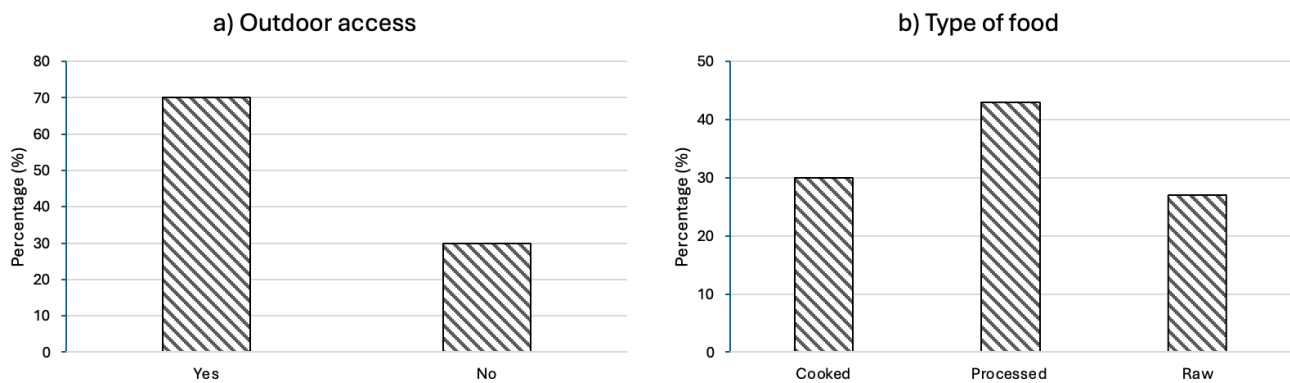


Figure 3. Outdoor access and type of food fed to cats: Cooked, Processed and Raw.

3.3. Litter Box Usage

Litter box usage was reported for ($n = 55$, 66.3%) of cats, with a litter box but no litter material ($n = 30$, 36.1%) being the most common (**Figure 4**).

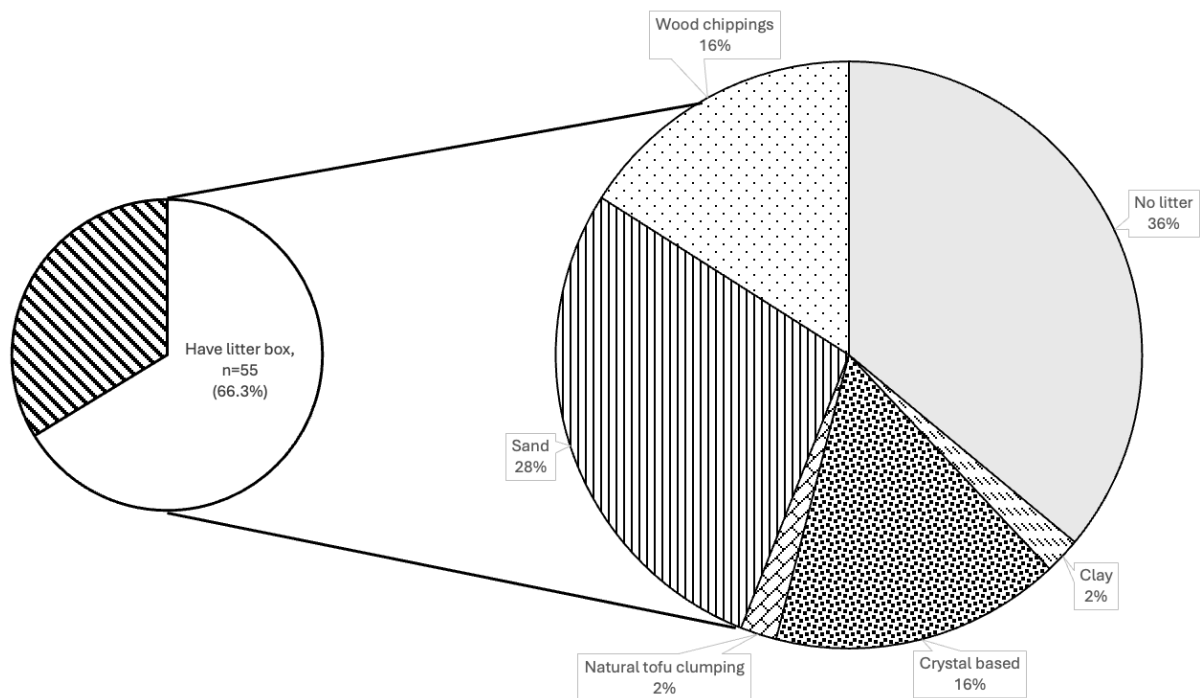


Figure 4. Litter box usage of cats brought to small animal clinics in Kampala.

3.4. Seroprevalence of Toxoplasmosis in Domestic Cats Brought to Small Animal Clinics in Kampala District

Out of the 80 cats tested, the true prevalence was estimated at 45.2% as shown in **Figure 5**.

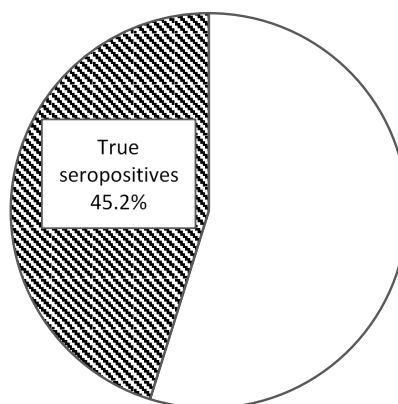


Figure 5. Graph true seroprevalence of Toxoplasmosis in domestic cats brought to small animal clinics in Kampala at a 95% Confidence Intervals.

3.5. Risk Factors of Toxoplasmosis

The bivariate analysis revealed several screening variables ($p \leq 0.25$) for multivariable analysis. These included: adult or senior cats (OR 6.94, 95% CI: 2.60 - 18.54, <0.001), intact sterilization status (OR 1.90, 95% CI: 0.80 - 4.53, 0.153), cats originating from the street (OR 2.71, 95% CI: 1.02 - 7.15, 0.045), presence of other cats (OR 3.09, 95% CI: 1.26 - 7.59, 0.014) or animals (OR 2.91, 95% CI: 1.13 - 7.50, 0.027) at home, feeding cats raw food (OR 7.07, 95% CI: 1.98 - 25.28, 0.003) and outdoor access (OR 11.67, 95% CI: 3.50 - 38.94, <0.001).

For the owners, it was found that owners with a primary or no education level (OR 2.10, 95% CI: 0.80 - 5.49, 0.130) and owners who live in either bungalows or grass thatched houses (OR 12.9, 95% CI: 1.552 - 107.25, 0.018) owned cats with a higher chance of being infected with Toxoplasmosis. Of these screening variables, only raw food (aOR 6.76 95% CI: 1.38 - 33.12, 0.018), roaming outdoors (aOR 6.20 95% CI: 1.54 - 24.95, 0.010) and adult/senior age group (aOR 3.82, 95% CI: 1.20 - 12.20, 0.023) remained significantly associated with Toxoplasmosis seropositivity after multivariable analysis. Roaming outdoors was significantly associated with bungalow/hut housing (aOR 10.23, 95% CI: 1.97 - 52.99, 0.006) and having other cats at home (aOR 4.37, 95% CI: 1.41 - 13.61, 0.011).

4. Discussion

4.1. Seroprevalence of Toxoplasmosis in Cats

The study investigated the seroprevalence of *T. gondii* among domestic cats presented to small animal clinics in Kampala district and assessed associated risk factors. The true seroprevalence was 45.2%, indicating that exposure to *T. gondii* is common in the study population. This relatively high seroprevalence may be associated to factors such as age, sterilization status, origin of cat, cohabitation with other animals, raw meat feeding and outdoor access, all of which increase the likelihood of infection.

The seroprevalence observed in this study was lower than the 91.6% reported in Ethiopia nonetheless it still indicates substantial exposure [25]. The compara-

tively high prevalence reported in both settings may reflect similar environmental and management conditions common in many urban and peri-urban areas of East Africa, including warm climatic conditions that favour survival of *T. gondii* oocysts [26], and opportunities for hunting by domestic cats [27]. In contrast, a study from Kenya reported a prevalence of 7.8%. This was likely an underestimation since they relied on faecal detection which identified only actively shedding cats.

Because this study did not distinguish between IgG and IgM antibodies, this restricted our ability to accurately identify active infections and therefore potential shedders of oocysts. Accordingly, these results should be interpreted as evidence of widespread prior exposure rather than ongoing transmission or current oocyst shedding. Nevertheless, the high seroprevalence suggests substantial environmental contamination and/or frequent exposure to infection sources in Kampala. These findings underscore the need for public health education promoting measures to reduce infection risk, including limiting predation, avoiding raw meat feeding, and improving hygiene practices to minimize environmental contamination with *T. gondii* oocysts.

4.2. Risk Factors of Toxoplasmosis in Cats

The most significant risk factor of Toxoplasmosis in our study was the consumption of raw or uncooked food. This finding is consistent with previous studies, which have shown that ingestion of raw food increases the likelihood of exposure to infective tissue cysts that would otherwise be destroyed through cooking [16] [28]–[30]. In our study, the association suggests that the food sources commonly provided to cats may have been contaminated with infective tissue cysts. These results underscore the importance of improving feeding practices, particularly by discouraging the provision of raw meat to cats, as well as increasing awareness among pet owners about the associated risks.

Outdoor access was also significantly associated with *T. gondii* seropositivity in cats in this study. Cats allowed to roam outdoors had a higher likelihood of exposure to infection potentially due to increased opportunities to hunt and consume infected prey, such as rodents and birds, which may harbour tissue cysts. In addition, free-roaming cats are more likely to come into contact with contaminated soil or water containing infective *T. gondii* oocysts shed by other cats.

Cats from multi-cat households were more likely to have outdoor access compared to cats kept individually. This pattern may reflect owner management practices rather than the cats' behavioural preference to roam. Owners managing multiple cats may face difficulties maintaining strict indoor confinement and adequate sanitation, including litter box management, odour control, and limited indoor space. As a result, some owners may permit outdoor access as a practical measure to ease management constraints or reduce indoor overcrowding.

Similarly, cats living in bungalows or huts were more likely to roam freely compared to those in apartments. This difference is likely influenced by housing structure and pet management practices. Apartments typically have limited exit points,

restricted outdoor access, and housing regulations that encourage keeping pets indoors. In contrast, bungalows and huts often feature open compounds or outdoor spaces that facilitate outdoor access and make it more difficult to fully restrict cats' movement. These findings suggest that both housing environment and owner management practices play important roles in determining the extent of outdoor exposure among domestic cats.

Furthermore, adult and senior cats were more likely to be seropositive than younger cats. This finding is consistent with previous studies, which have shown that the likelihood of exposure to infective tissue cysts in meat increases with age [16] [30] [31]. However, some studies have reported higher seroprevalences in young cats (around 2 months old) but this is attributed to maternal antibodies transferred via colostrum from seropositive queens. These antibodies typically decline and disappear by approximately 12 weeks of age [32].

Other factors associated with Toxoplasmosis seropositivity in the bivariate analysis included intact sterilization status. This association is likely attributable to behavioural differences, particularly those related to mating and territorial activity [31]. Intact cats, driven by hormonal influences, tend to engage in more extensive roaming and hunting behaviours, which in turn elevate their exposure to infected prey and contaminated environments [33].

Although sterilization has been shown to reduce the risk of Toxoplasmosis in cats [15] [34], its uptake in pet owners in Uganda remains low. This is largely due to limited awareness, financial constraints and certain beliefs that animals should be allowed to express natural behaviours such as mating and reproduction [23]. This highlights the need for targeted community sensitization and educational initiatives to promote sterilization, not only as a population control measure but also as a strategy to reduce exposure to infections.

Cats adopted from the streets showed a higher seropositivity rate (77.8%), although this relationship was not statistically significant. It is plausible that stray cats, due to their scavenging and hunting behaviour and consumption of wild prey, are at a higher risk of exposure to *T. gondii*. Indeed, very high seroprevalence rates, upwards of 95% have been reported in stray and feral cats in Egypt [35].

Owner education level was linked to *T. gondii* seropositivity among cats in this study. Cats owned by individuals with only primary or no formal education were more likely to be seropositive for *T. gondii* compared to those owned by individuals with secondary or tertiary education. This association may reflect differences in awareness and knowledge of recommended cat management and feeding practices, particularly the risks associated with feeding raw or undercooked meat to cats.

In addition to education level, socioeconomic status may also influence cat management practices. Owners with limited financial resources may be less able to consistently provide commercially prepared, or properly cooked food for their cats. Consequently, cats may more frequently be fed raw leftovers or allowed unrestricted outdoor access to hunt for their own food, thereby increasing opportu-

nities for ingestion of infected prey or exposure to environments contaminated with *T. gondii* oocysts.

Although this study provides valuable insights into the seroprevalence of *T. gondii* in domestic cats, it was limited to animals presented at veterinary clinics. Consequently, the findings may not fully represent the broader cat population, particularly free-roaming or rural cats that rarely access veterinary services. Future research should include cats from diverse settings, from households without veterinary access, stray populations, and rural communities. Such studies would provide a more comprehensive understanding of infection dynamics, associated risk factors, and their potential public health implications.

5. Conclusion

This study identified a high seroprevalence (45.2%) of *T. gondii* among domestic cats in Kampala, indicating a potential public health concern. Significant risk factors included consumption of raw meat, outdoor access, and increasing age. Roaming behaviour was more frequently observed in cats from bungalow-type residences and multi-cat households. Stray cats and those from households with lower levels of owner education also, demonstrated higher exposure rates. These findings highlight the importance of owner education on safe feeding practices, appropriate litter box management, and sterilization to reduce environmental contamination and transmission risk. Although the study was limited to clinic-presented cats, it provides useful insights for targeted control measures. Future studies should include free-roaming and rural cat populations to better elucidate infection dynamics and support the development of comprehensive prevention strategies.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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