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Detection of *IgG antibodies* against *T. gondii* and associated risk factors in domestic and stray cats in Lusaka District, Zambia

Farai phiri [1],*, Careen Hankanga [1], Ntombi Mudenda [1], Ngonda Saasa[2], Mukubesa Andrew Nalishuwa[2].

1Department of Clinical Studies, School of Veterinary Medicine, University of Zambia.
PO Box 32379, Lusaka, Zambia.:
2 Department of Disease Control, School of Veterinary Medicine, University of Zambia,
PO Box 32379, Lusaka, Zambia.:
*Corresponding Author and address: Farai Phiri, Department of Clinical Studies, School of Veterinary Medicine, University of Zambia. Po box 32379, Lusaka, Zambia. Email: farai.phiri@unza.zm.

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ABSTRACT

Toxoplasma gondii (T. gondii) is a nearly ever-present organism that infects humans, wildlife, birds, domestic and food animals. *T. gondii* has been reported in pigs, chickens, sheep, goats, and cattle (1). However, only members of the cat family (*Felidae*) are the definitive host and shed the environmentally resistant oocyst form of the organism in their faeces. Transmission to humans usually occurs by ingesting cysts in undercooked meat and exposure to soil water which is contaminated by oocysts (2). Feline infections are typically subclinical; congenitally infected kittens are the ones that are most likely to have clinical signs of infection, but previously clinically healthy adult cats may also be affected (3).

Despite the disease's significance in public health, very few studies have been done on *T. gondii* in Zambia. This study was a cross-sectional survey to investigate the seroprevalence and risk factors of Toxoplasmosis. A survey was carried out using a questionnaire that was distributed to cat owners who attended the veterinary clinics. The questionnaire had demographic data for the cats, questions related to the risk factors of Toxoplasmosis in cats, and practices surrounding the cat owners, like how they handle cat litter, whether their cat was indoor or outdoor, contact of the cat with other cats, and straying behaviour of their cat. A total of 178 blood samples were collected from both stray and domestic cats in this study. Of the 178 samples tested for *T. gondii IgG antibodies*, 88 were from domestic cats and 90 from stray cats. 30 out of 178 samples were positive for antibodies, indicating an overall seroprevalence of 16.85%. Of the 30 positive seropositive samples, 60% (18) came from domestic cats and 40% (12) from stray cats. The seroprevalence for domestic cats was found to be 20.4% and that of strays 13.3%. The two risk factors found to be associated with *toxoplasma* Seropositivity were sex (p=0.007, 95%, CI 0.071,0.976) and diet (p=0.038, 95%, CI 1.395,95.418)

It was concluded that *Toxoplasma gondii* was widely spread in the study population. The presence of the antibodies indicated that at one point in their lives, these cats were actively infected and shedding oocysts in the environment, thereby posing a risk to humans and other species like sheep and chickens.

KEYSWORDS: *Toxoplasmosis, Toxoplasma gondii, Seropositivity, seropositive, antibodies, zoonotic, transmission.*

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a nearly ubiquitous organism that infects humans, wildlife, birds, domestic and food animals. *T. gondii* has been reported in pigs, chickens, sheep, goats, and cattle (1). However, only members of the cat family (*Felidae*) are the definitive host and shed the environmentally resistant oocyst form of the organism in their faeces. Transmission usually occurs by ingesting cysts in undercooked meat and exposure to soil and water contaminated by oocysts (2). The disease is of public health importance because of the economic loss caused by food animals and the severe disease it causes in foetuses through their pregnant mothers and immune-compromised individuals.

The life cycle starts with a domestic cat that eats a mouse that is infected with *T. gondii* in the muscle as a cyst (4). The parasite persists and passes to the stomach, where it infects the epithelial cells

of the cat's small intestines. The parasite undergoes sexual development and reproduces many zygotes that contain oocysts. The infected cats' epithelial cells burst and release the oocysts in their faeces (5). Intermediate hosts (other animal species like birds and rodents) acquire the infection from ingesting oocyst-contaminated soil, water, and plant materials (6).

Most feline infections occur postnatally through ingesting infected tissue cysts or, rarely, oocysts, although congenital infections can occur. Feline infections are typically subclinical; congenitally infected kittens are the most likely to have clinical signs of infection, but previously clinically healthy adult cats may also be affected (3). Cats are more likely to shed oocysts following ingestion of tissue cysts rather than tachyzoites or oocysts. Ingesting only one bradyzoite will lead to feline infection compared to 1000 oocysts that a cat has to ingest to develop an infection.

Feline infections are typically subclinical; congenitally infected kittens are the ones most likely to have clinical signs of infection, but previously clinically healthy adult cats may also be affected (3). Common symptoms of *T. gondii* infections in cats include fever, ocular inflammation, anorexia, lethargy, abdominal discomfort and neurological abnormalities. Hepatitis, cholangiohepatitis, pneumonia, and encephalitis with concurrent signs of ascites, lethargy and dyspnea have been seen in trans-placentally infected kittens as well (7). Clinical signs of adult cats are non-specific (8). Inflammatory bowel disease (9) and regional lymphadenopathy (10,11) have also been recorded in cats with T. gondii. Hepatic disease has also been reported in cats with Toxoplasmosis (10,12).

Risk factors for Toxoplasmosis in cats include sex, age, body condition score, diet, access to hunting (feeding raw meat), and the number of cats in the household. They can be analysed using multivariate methods (13,14,15). Cat owners can reduce the risk of exposure for their pets by keeping them indoors and avoiding feeding them raw or undercooked meat to enter the environment and later be transmitted to humans (16). Other than domestic cats responsible for infecting the environment, there are large populations of unowned cats worldwide (16). This is a public nuisance, especially if the number of strays is very big. The social and economic impact Toxoplasmosis has on the public is huge, because it causes human suffering and the financial burden of caring for sick children with blindness and mental retardation and illness (17,18). Feline toxoplasmosis has proven to be very difficult to diagnose, and although there are a number of changes that occur under radiography, haematology and biochemistry, these are, however, not pathognomonic (19). Several diagnostic methods have been used worldwide to determine *T. gondii* infection; these include serology and molecular. Although a single positive IgG titer indicates exposure, clinical Toxoplasmosis is indicated by a positive IgM titer or a fourfold increase in the IgG levels in paired serum samples taken 2-4 weeks apart (3) because most cats seroconvert after they have finished shedding oocysts only at a single point in their lifetime. Antigen detection has also proven useful in detecting *T. gondii* in recently sub-clinically infected cats (19).

This study determined the seroprevalence of *Toxoplasma gondii* and investigated its associated risk factors in cats in the Lusaka District of Zambia.

MATERIALS AND METHODS

Study Design and Study Area

A cross-sectional study was purposively conducted in the Lusaka District of the Lusaka Province of Zambia. The district has a total area of 418km² and an altitude of 1,279m. It is located at 15° 24'59.99''S and 29° 00'0.00''E. The study focused on domestic and stray cats of any age, sex, and age. The exclusion criteria were all critically ill cats. The study was done in compliance with ERES Converge research and ethics committee guidelines.

Sample Size and Sampling Technique

One hundred and seventy-eight blood samples were randomly collected from cats presented at three selected veterinary clinics, which were conveniently selected, whilst stray cats were trapped in different areas around Lusaka District as shown in Figure 1. 90 samples were conveniently collected from stray cats, and 88 were collected from domestic cats. At the time of sampling from domestic cats, consent was sought from the cat's owners. A millilitre of blood was drawn from the jugular or cephalic veins into a plain serum tube. The serum was later separated by centrifugation at 1000 rpm and stored at -20°C after labelling. A questionnaire was then administered focusing on possible risk factors, such as sex, age, diet, contact with other cats, habitation behaviour, straying behaviour, and manure management.

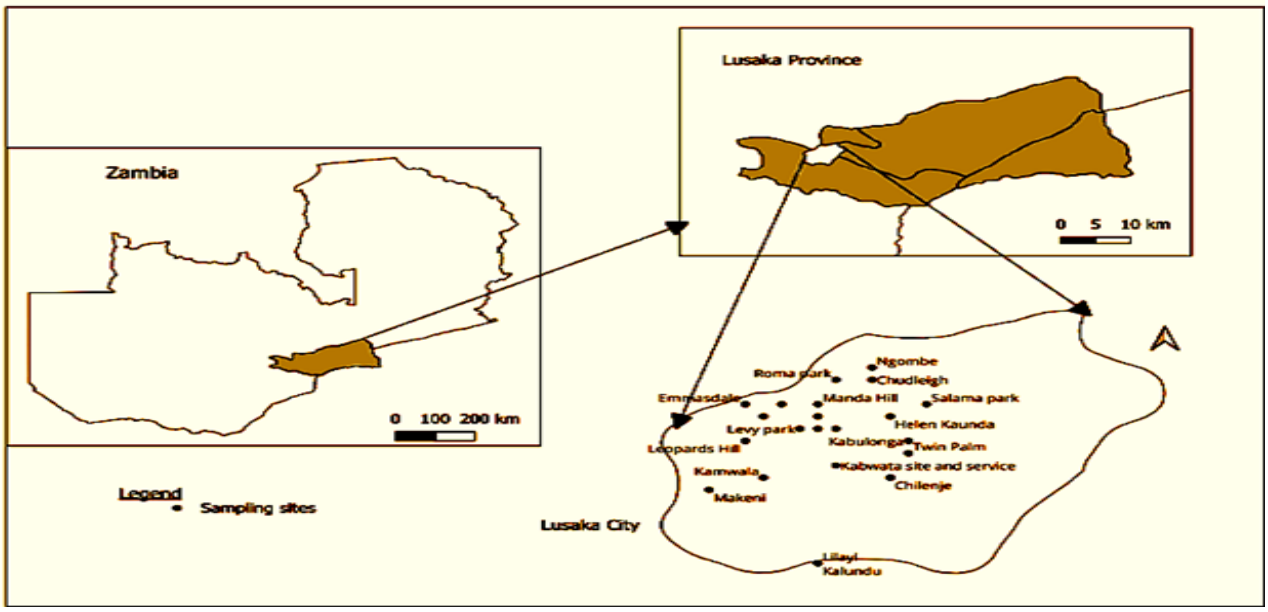

Figure 1: Map of Lusaka showing residential information of cat owners in this study (Generated by authors).

Sero-diagnosis (Indirect ELISA)

Indirect multispecies ELISA kit (indirect ELISA assay (ID screen® toxoplasmosis-indirect Multispecies, IDVET, France) was used to detect the antibodies of *T. gondii* in blood (serum) against *Toxoplasma gondii* p30 protein. The procedure was followed according to the manufacturer's instructions. The serum was thawed until defrosted. 90µl of diluted buffer 2 was added to each micro well and 10µl of the negative control was added to A1 and B1 wells. Another 10µl of the positive control was added to C1 and D1 wells. Thereafter, ten microliters of the serum sample were added to the remaining micro wells. The plate was then incubated for 45 minutes at 21ºC for 4 days. Each well was then emptied and washed with 300µl of wash solution. Then 100µl of substrate solution was added to each well and the plate was incubated for 15minutes at 21ºC in the dark. After this, 100µl of the stop solution was then added to each well to stop the reaction. The results were then read and validated using the negative and positive control provided for by the manufacturer at 450nm. The samples were considered positive if the OD exceeded or was equal to 50%, doubtful if between 40 and 50% and considered negative if below 40 %.the formula used was;

% = 100*OD of sample/OD of positive control (where OD is the Optic Density and PC is Positive Control)

Questionnaire Survey

Data and sample collection were done between 2018 and 2019. A questionnaire survey was carried out during sample collection from domestic cats. Owners were interviewed through a questionnaire to assess the risk factors for toxoplasmosis in cats. For domestic cats, the inclusion criteria were all cats that were not very ill and were coming from within Lusaka District and if the cats were too small or too sick, they were not included in the study. The questionnaire was meant to assess the risk factors like age, sex, whether the cat was indoor or outdoor, contact with other cats, straying, and diet. There was no questionnaire for stray cats, however, a data sheet was available. Age of the strays was determined by estimation. The data collected was organised in excel and analysed using chi-square and binary regress in Statistical Package for Social Sciences (SPSS).

RESULTS

Seroprevalence

Of the 178 samples tested, 88 were from domestic cats and 90 from stray cats. Of these 30 (16.85%) were sero-positive for *Toxoplasma* IgG. From the 30 seropositive samples that tested positive, 60% (18) were domestic cats and 40% (12) were stray cats. The overall seroprevalence in both domestic and stray cats was found to be 16.85%. The seroprevalence by source of sample was 20.4% for domestic cats and 13.3% for stray cats.

Proportions of seropositivity

Table 1 shows the proportions of seropositivity according to the different variables, this was obtained using the chi-square.

Table 1: Proportions of seropositivity according to variables

Variables	Description	Negatives	Positives	Total
Sample Origin				
Stray		73	12	85
Domestic		46	18	64
Sex				
Male		49	18	67
Female		70	12	82
Contact with others				
Yes		95	24	119
No		21	6	27
Missing		3	0	3
Passing of stool				
Missing		3	0	3
Litterbox		11	4	15
Outside		105	26	131
Habitation				
Missing		3	0	3
Indoor		15	2	17
Outdoor		86	20	106
Both indoor and outdoor		15	8	23
Straying behavior				
Missing		9	1	10
Yes		90	22	112
No		20	7	27

Risk factors of *toxoplasma gondii* infection in cats

The variables used to determine the risk factors associated with toxoplasmosis in cats were, sex, age, diet, housing (indoor or outdoor), owner's awareness to toxoplasmosis, straying behaviour, presence of other cats or animals in the same premise, and contact with other cats. Binary Logistic Regression model that was used to determine the risk factors in this study.

From the regression analysis, only eating other diets was found to be a predictor variable with a P value of 0.025 and a confidence interval of 1.550-616.35 at 95% (shown in Table 2). It was established that older cats were more likely to get *T. gondii* infection than younger cats. As the cat got older, the chances of getting infected became higher.

Table 2: Binary logistic regression of risk factors

	B(estimated logit coefficient)	S.E.(standard error of coefficient)	Wald	df	Sig.(significant level of coefficient)	Exp(B),odds ratio	95% C.I.for EXP(B) Lower	Upper
Sex(1)	-.929	.938	.982	1	.322	.395	.063	2.480
Age			1.858	4	.762			
Age(1)	-.465	41497.106	.000	1	1.000	.628	.000	.
Age(2)	16.122	40192.979	.000	1	1.000	10037337.447	.000	.
Age(3)	19.336	40192.979	.000	1	1.000	249789355.695	.000	.
Age(4)	18.313	40192.979	.000	1	1.000	89771624.299	.000	.
Diet_raw_meat	-3.254	2.307	1.989	1	.158	.039	.000	3.553
Diet_other	3.431	1.527	5.048	1	.025	30.905	1.550	616.353
Do_they_live_indoor_outdoor	.643	.708	.825	1	.364	1.902	.475	7.618
Pass_stool	-1.819	1.406	1.675	1	.196	.162	.010	2.549
Contact_with_others	-.045	.913	.002	1	.960	.956	.160	5.719
Vaccinated	.942	1.097	.737	1	.390	2.566	.299	22.040
Constant	-18.575	40192.980	.000	1	1.000	.000		

Variable(s) entered : Sex, Age, Diet_raw_meat, Diet_other, Do_they_live_indoor_outdoor, Pass_stool, Contact_with_others,

Chi-square was used to test the association between the risk factors (variables) and the outcome (positivity). Only 149 samples were considered valid in the chi square analysis. However, since the stray cats did not have a questionnaire only the domestic cats were analysed to determine the risk factors.

Two variables were found to be significantly associated with seropositivity. The variables included eating other foods (P value of 0.007) and sex with a P value of 0.038. This is shown in Table 3 below.

Table 3: Association between the risk factors

Variable	Chi-square value	DF	P VALUE	95% C,I
Contact with others	0.395	2	0.530	0.422-5.338
Excretion (where they pass stool)	1.547	1	0.214	0.103-1.696
Habitation (Indoor/ outdoor)	1.605	1	0.205	0.079-2.007
Straying behavior (leaving the yard or not)	1.310	1	0.252	0.434-31.387
Consumption of raw meat	0.058	1	0.810	0.072-7.864
Consumption of kitchen food	0.395	1	0.530	0.187-2.372
Consumption of commercial food	0.207	1	0.649	0.221-2.567
Consumption of other diet/foods	7.335	1	0.007	1.395-95.418
Sex	4.304	1	0.038	0.071-0.976
Age	7.942	4	0.094	
Presence of other cats	2.594	1	0.107	0.768-10.949
Presence of other animal species	1.121	1	0.290	
Neutered	1.121	1	0.290	0.148-1.785
Vaccination	1.501	1	0.221	0.118-1.664
Disposal of fecal material	0.318	1	0.573	0.207-2.390

When the strays were compared with the domestic cats, sample source was significantly associated with seropositivity with a p value of 0.035 and there was a significant difference between the two groups (Table 4).

Table 4: Measure of the association between sample source and seropositivity

	Chi value	DF	to	Comment
Sample source (domestic, stray)	4.455	1	0.035	significant

DISCUSSION

This study investigated the seroprevalence of *T. gondii* and its associated risk factors. The overall seroprevalence of both domestic and stray cats was found to be 16.85%. This study indicates that the seroprevalence of Toxoplasmosis in cats in Lusaka is much lower than in South Africa at 37.1% (20). Other *T. gondii* serological studies conducted in Thailand and Egypt revealed a 15.4% and 97.4% prevalence, respectively (21,22). It is evident that the results of this study are similar to those of Thailand; this could be due to similar climates in the two regions (tropical climate). The difference in seropositivity with other regions could be attributed to different environmental conditions because it has been established that *T. gondii* is most prevalent in hot and humid regions (23).

The current study recorded a higher seropositivity in domestic cats compared to the stray cat population. The reason for this could be attributed to the way cats are kept in Zambia. Of the domestic cats that were positive (18 cats), 11.11% (2) cats were kept strictly indoors, 44.44% (8) cats were kept outdoors, and the rest 44.44% (8) cats lived and moved between indoors and outdoors. This shows that the majority are kept partly or entirely outdoors, increasing exposure time in the environment and exposure load. This suggests that most domestic cats are constantly in contact with the environment, like strays or feral cats. Evidence shows that about 14 to 36% of cats are acquired as strays (24). Likewise, a good number of stray cats in Lusaka are previously owned cats that are later

re-homed after being rescued by animal welfare societies in Lusaka District. There is a huge overlap in husbandry practices between the domestic and stray cats here in Lusaka District. The environment is contaminated with *T. gondii* oocysts, exposing domestic cats to the dangers of going outside. Environmental contamination could also partly explain the detected antibodies in humans in a study done by Frimpong *et al*. (25) in Lusaka District. It's either the humans come in contact with the infected cats, or they come in contact with a contaminated environment. Must *et al*. (26) already established that environmental contamination with *T. gondii* oocyst poses a long-term risk to other hosts, including humans.

This study established that sex was associated with seropositivity. There was a significant difference between the males and females, with a p-value of 0.038 (CI, 95%). In this study, there were more males with *IgG antibodies* (60%) than females (40%). This is similar to a study by Lee *et al*. (27) in Korea, where they found that the seropositivity in male strays was higher than that of females. The difference between males and females in this study could be attributed to the different behaviours of male and female cats, with males known to stray a lot more than female cats, thereby exposing themselves to *T. gondii* (27). However, this finding in this study is different from a study by Lopez *et al*. (28), who established a similarity in seropositivity between the males and females.

The current study established that older cats were more likely to be infected with *T. gondii* infection than younger cats. This is similar to a study by Must *et al*. (26), who found that cats older than one year old were 8.7 times more likely to test seropositive than younger cats, a clear indication that these cats get exposed to age. This supports several authors like Opsteegh *et al*. (29), who found that infection in older cats is due to continuous exposure to *T. gondii* in the environment with time. Other authors have also shown that in several hosts, domestic cats inclusive, the seropositivity of *T. gondii* infections increases with age, indicating that most of these infections are postnatally acquired (5). Castillo-Morales *et al*. (13), however, had different findings from those in this study, whose study showed that infection by *T. gondii* was high in younger cats, and this was attributed to the weak immunity that young stray undernourished kittens have. The other high odds ratio from the age groups could be due to the low numbers of cats in the said groups and not due to an increased risk.

The risk factors associated with the seropositivity of *toxoplasma* were found to be sex and diet, which is contrary to results found by Tagwireyi *et al*. (23), who found the risk factor to be age in cats. Besné-Mérida *et al*. (14), however, found risk factors to be sex and the frequent consumption of raw meat. There was also no significant association between sex or diet with toxoplasmosis infection in a study by Castillo-Morales *et al*. (13). Other factors like age and outdoor access were found to be associated with *toxoplasma* infection; this was revealed in a study done by Deske *et al*. (30). One study showed that those who ate raw meat had a high risk of getting an infection (13,14). This differs from this study, which indicates a high risk in cats fed on other foods.

CONCLUSION AND RECOMMENDATIONS.

To the best knowledge of the researcher, this study is the first report on *T. gondii* in cats in Lusaka District, Zambia, and it showed the presence of *T. gondii* antibodies (both in stray and domestic cats with a seroprevalence of 16.85%. Of these, 60% were domestic cats, and 40% were stray cats, and there was a significant difference between the two groups. The risk factors found in this study were sex and diet. This information is important to ensure public awareness of the importance of hygiene practices when handling cats. It is vital not only for the communities that harbour cats but also for policymakers on the control of cat populations and, in particular, strays.

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