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Seroprevalence and associated risk factors of *Toxoplasma gondii* in cats from selected areas in urban Harare, Zimbabwe

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Abstract. A cross-sectional study was carried out from September 2017 to May 2018 to determine the seroprevalence and associated risk factors for *Toxoplasma gondii* infection in cats from selected areas of urban Harare. Serum samples from 140 domestic cats were screened for the presence of *Toxoplasma gondii* p30 antigen antibodies using an indirect enzyme-linked immunosorbent assay (ELISA) kit. Faecal flotation to determine oocyst shedding was also done on faeces of 21 of the bled cats. Selected demographic and other data on cat lifestyle were obtained using a structured questionnaire and evaluated for their potential as risk factors for *T. gondii* infection. Overall, of the 140 serum samples tested, 77 (55%) tested positive (n = 140; 95% CI= 0.47-0.63), with the highest seroprevalence (78.7%) in cats that had free access to outdoor activity. The risk factors that were found to have a significant association with *T. gondii* seropositivity included outdoor access/activity (p = 0.006, χ^2= 7.4228, Fisher's test = 0.009), age (p = 0.036, χ^2 = 6.6544, Fisher's test = 0.037) and sex (p = 0.032, χ^2 = 4.6, Fisher's test = 0.041). No *T. gondii* oocysts were detected in the faecal samples. It was concluded that the seroprevalence of *T. gondii* in the sampled cats was high and outdoor access increased prevalence significantly. There is need for public awareness on the risk factors and potential effects of *T. gondii* infection in cats, other animals including man and the development of infection monitoring methods.

Keywords: *Toxoplasma gondii;* Seropositivity; Risk factors; Domestic cats; Urban Harare.

Seroprevalenţa şi factorii de risc asociaţi ai *Toxoplasma gondii* la pisici din zone selectate din oraşul Harare, Zimbabwe

Rezumat. Un studiu transversal a fost efectuat în perioada septembrie 2017 până în mai 2018 pentru a determina seroprevalenţa şi factorii de risc asociaţi pentru infecţia cu *Toxoplasma gondii* la pisici din zonele

selectate ale oraşului Harare. Probele de ser de la 140 de pisici domestice au fost testate pentru prezenţa anticorpilor antigen *Toxoplasma gondii* p30 utilizând un kit de testare imunosorbantă indirectă legată de enzime (ELISA). Flotaţia fecală pentru a determina eliminarea oochistului a fost făcută şi pe fecalele a 21 dintre pisicile sângerate. Datele demografice şi alte date ale stilului de viaţă ale pisicilor au fost obţinute folosind un chestionar structurat şi au fost evaluate pentru potenţialul lor ca factori de risc pentru infecţia cu *T. gondii.* În general, dintre cele 140 de probe de ser testate, 77 (55%) au fost testate pozitiv (n = 140; 95% CI= 0,47-0,63), cu cea mai mare seroprevalenţă (78,7%) la pisicile care au avut acces liber la activităţi în aer liber. Factorii de risc care s-au dovedit a avea o asociere semnificativă cu seropozitivitatea *T. gondii* au inclus accesul/activitatea în aer liber (p = 0,006, χ2= 7,4228, testul Fisher = 0,009), vârsta (p = 0,036, χ2 = 6,6544, testul Fisher = 0.037) şi sex (p = 0,032, χ2 = 4,6, testul lui Fisher = 0,041). Nu au fost detectate oochisturi de *T. gondii* în probele de fecale. S-a ajuns la concluzia că seroprevalenţa *T. gondii* la pisicile eşantionate a fost ridicată, iar accesul în aer liber a crescut semnificativ prevalenţa. Este nevoie de conştientizarea publicului cu privire la factorii de risc şi efectele potenţiale ale infecţiei cu *T. gondii* la pisici, alte animale, inclusiv la om şi dezvoltarea metodelor de monitorizare a infecţiei.

Cuvinte cheie: *Toxoplasma gondii;* Seropozitivitate; Factori de risc; Pisici domestice; Harare.

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Introduction

Toxoplasma gondii is a cosmopolitan zoonotic coccidian parasite which infects most, if not all, species of birds and mammals (Tenter et al., 2000). It is an intracellular parasite which has capacity to multiply tremendously in the newly infected and/or hosts with incompetent immune systems, resulting in host cell destruction and clinical disease (Dubey, 2009). The only known definitive hosts for the parasite are domestic and wild Felidae whilst all warm-blooded animals, including felids at initial exposure, serve as intermediate hosts (Dubey, 2008).

The majority of *Toxoplasma* infections in immune competent intermediate hosts are either mild or asymptomatic. Congenital infections in kittens tend to cause more serious toxoplasmosis than reactivation of latent infections in older cats (Elmore et al., 2010), but acute toxoplasmosis may present in immunocompromised older cats (Dubey and Lappin, 2006). Transplacentally or lactogenically infected kittens develop more severe signs and frequently die of pulmonary or hepatic disease (Dubey and Lappin, 2006). In a study in South Africa, cats positive for *T. gondii* IgM had shown elevated liver enzymes (Lobetti and Lappin, 2012), mostly likely due to underlying hepatic disease which has been documented in toxoplasmosis in cats.

The protozoa exist in three forms, namely sporozoite, tachyzoite and bradyzoite. Sporozoites are formed in the environment within oocysts excreted in faeces of the definitive hosts whilst tachyzoites and bradyzoite cysts are found within extra-intestinal tissue of intermediate hosts (Tenter et al., 2000). The modes of transmission to the intermediate hosts are the faeco-oral route (via oocysts in feline faeces), transplacental via the tachyzoite and by ingestion of infected meat and milk with tissue cysts (Dubey, 2009). The oocyst is small ranging between 11-13µm in diameter, unsporulated (non-infective) when passed in faeces and sporulates and becomes infective in about 1-5 days (average of 3 days) (Dubey, 1998).

Felids are considered a primary source of *T. gondii* infection for human beings and other domestic animals because they are the definitive hosts that can excrete

environmentally-resistant, infective oocysts in their faeces which when ingested by warm-blooded animals eventually develop to infective tissue cysts (Dubey, 2009; Elmore et al., 2010). Even though the final host excretes oocysts for a short period of time (only 1–2 weeks), millions may be excreted. Oocysts can survive in the environment for several months and are noticeably resistant to freezing, drying and conventional disinfectants, but are not heat-resistant and are destroyed at 70°C for 10 min (Dubey and Beattie, 1988; Dubey and Jones, 2008). The oocyst stage contaminating pasture and feed is likely to be the most important source of infection for livestock. It is also reported that oocyst-induced infections tend to be more severe in the intermediate hosts than the tissue-cyst-induced ones (Dubey and Beattie, 1988).

Seroprevalences in food animals (chickens, pigs, sheep and goats) reared extensively and/or under low biosecurity in Zimbabwe have been high (Hove et al., 2005a; 2005b). However, little is known about the seroepidemiology of *T. gondii* in cats in Southern Africa. Very high seroprevalences (Range: 86-100%) of *T. gondii* were detected in free-range wild leopards and lions from South Africa, Botswana and Zimbabwe (Penzhorn et al., 2002; Hove and Mukaratirwa, 2005). Studies in domestic cats have indicated a range of seroprevalences (IgG antibodies), from low for cats under good care, with little access to raw or undercooked meat (Lopes et al., 2017) to high in feral (Hammond-Aryee et al., 2015) and domestic cats with free access to the outdoors (Tagwirei et al., 2019). Knowledge of the epidemiology of *T. gondii* in domestic cats from Zimbabwe is a step towards the control of its spread to other animals and humans, especially pregnant woman and immunocompromised individuals. Thus, the aim of the study was to determine the seroprevalence and associated risk factors for *T. gondii* infection in domestic cats of urban Harare.

Materials and methods

Study areas

The study was performed between September 2017 and April 2018 in urban Harare on cats presented at seven randomly selected veterinary surgeries (50), resident at two cat shelters (63) and sampled from homes (27). Participation was based on the management's willingness to allow sampling and the owners' consent.

Cat shelters provide various veterinary care to communities and animal support. They also house cats rescued from the streets and from irresponsible cat owners, which are made available for adoption after a period of quarantine. The grounds at shelters were covered with grass and some trees, with several enclosures for the animals surrounded by some concrete yards. The perimeter was delimited by high fences with aluminium sheeting in the upper portion to ensure that the animals would not escape. A larger proportion of cats that has been resident in the shelters for a longer period are however allowed access to the outdoor environment. They can roam freely around the property with access to soil and other animals during the day and are housed at night. Sheltered animals received manufactured dry feed, donated meat by meat processors and potable water. All cats received anti-rabies and cat flu vaccines annually and anthelmintic drugs sporadically. Food was available in feeders and drinking water was put in shallow troughs in the enclosures and also outside enclosures for those that are allowed to roam around freely. No litter boxes were available and hence the cats used the environment to defecate. Their faeces were sporadically collected from the by the keepers. The catchment areas for veterinary surgeries were from suburban Harare and cats from these areas had freedom to roam around. The cats from homes sampled from in the current study were fed on commercial feed as well as raw meat from local suppliers.

Epidemiological data collection

Epidemiological data on the risk factors thought to be associated with *T. gondii* seropositivity in cats was obtained using a questionnaire. The questionnaire captured data on age, sex, breed, neuter status and lifestyle (indoor versus outdoor activity) of the cats at any stage of their lives including access to soil and other animals.

Sample collection

Blood samples (1-2 ml) were collected from cats that were 3 months and above, by jugular venepuncture in plain blood tubes (Golden Vac-Tube, Zhejiang Gongdong Medical Technology Co., Ltd. China). The samples were sent to Diagnopath Veterinary Laboratory (Pvt, Ltd) under chilled conditions for processing. Clotted blood samples were spun in a centrifuge (Hettich Rotofix 32A benchtop centrifuge, Massachusetts, USA) at 3,000 rpm for 5 minutes. The serum from each sample was placed in an Eppendorf tube and stored at -20°C until testing.

Faecal samples were also collected from 21 of the bled cats and the samples were synchronized by assigning a unique matching identification (laboratory number) for each pair (serum sample and corresponding faecal sample). The faeces were either preserved in 10% formalin or stored in a refrigerator until testing.

Laboratory tests

Serology

Serological testing was carried out at Diagnopath Veterinary Laboratories (Pvt, Ltd) in Harare. An indirect ELISA which detects anti-*T. gondii* IgG antibodies to *T. gondii* P30 antigen in sera, plasma and meat juices (ID Screen® Toxoplasmosis Indirect Multi-species ELISA, ID.vet, Montpellier, France) was used. Testing and result validation were carried out as recommended by the manufacturer. A microplate reader was used to read the plates at 450 nm and the Optical Density (OD) was recorded for each sample. To enable uniformity of incubation times, the test was run in four batches until the sample size was completed.

The test was then validated using the mean OD value of the Positive Control (OD_{PC}). If OD_{PC} was >0.350 and the ratio of the mean OD values of the Positive and Negative Controls (OD_{PC}/OD_{NC}) was also >3), the test was considered valid. Interpretation of results was performed as follows. For each sample, the S/P percentage (S/P%) was calculated using the formula below:

$$S/P\,\% = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100$$

Serum samples with an S/P% of ≤40% were considered negative, the doubtful ones between 40% and 50% were also considered to be negative and those with ≥50% were considered positive.

Faecal flotation

All faecal flotations were carried out at the University of Zimbabwe Veterinary Parasitology Laboratory in the Para-clinical Department, Faculty of Veterinary Science. Three grams of faeces were weighed, homogenised in 42ml of water and sieved using a 0.15mm mesh. Of the filtrate, 15ml were collected and centrifuged at 1,500 rpm for 3 minutes. The supernatant was decanted, and the pellet was mixed with the super saturated salt solution (400g NaCl in 1 litre water) and allowed to stand for about 5 min. A McMaster chamber was then filled with the 0.15ml supersaturated salt/faeces mixture and examined under the microscope for *Toxoplasma* oocysts (Ministry of Agriculture, Fisheries and Food, 1986).

Statistical analysis

All data and results were captured in a Microsoft Excel work sheet and statistical analysis was performed using STATA® version SE 12.0 (StataCorp, College Station, Texas, USA). Selection of predictors in the regression model was done using the Chi-square test with values of $p < 0.05$ being considered significant.

Ethical considerations

Ethical approval was granted by the Ethical and Higher degrees committee of the Faculty of Veterinary Science, reference number 2018/10. The study purpose was explained to cat owners and shelter managers, who all agreed to participate and were allowed to withdraw the consent up to a week. Standard operating procedures were followed for sample collection from the cats.

Results

Descriptive statistics

Of the 140 sampled cats, the results indicated that 55% (n = 77; 95% CI: 0.47 - 0.63) were positive for IgG antibodies to the *T. gondii* p30 antigen in the ELISA. No oocysts were detected in the 21 faecal samples submitted for faecal flotation.

Bivariate analysis

The results of the bivariate analysis of the risk factors associated with the seroprevalence of *T. gondii* are shown in table 1. Sex, age and access to outdoor activity were significantly associated with *T. gondii* seropositivity (P<0.05). Location, household type, neuter status and faecal sample analysis (zero seropositivity) were not significantly associated with seropositivity to *T. gondii* (P>0.05).

Multivariable analysis

Multivariable logistic regression was carried out for the three risk factors (sex, age and access to outdoor activity) that showed a significant independent association (P<0.25) with *T. gondii* seropositivity in the univariable analysis. It showed that the cats that had access to outdoor activity had greatest odds of seropositivity to *T. gondii* (OR: 6) followed by male cats (OR: 3) (table 2).

The Hosmer- Lemeshow goodness-of-fit test showed that the model fit the data (χ^2 = 24.15, d.f. 7, P = 0.0011). The model had a good predictive ability (AUC = 0.78).

Table 1. Bivariate analysis of the risk factors associated with *T. gondii* seropositivity in domestic cats from selected areas of urban Harare (2017-2018)

Variable	Level	Bivariate analysis				
		N	Prevalence (%)	P	OR	CI (95%)
Location	0: Household	77	57.14	-	1.0	-
	1: Shelter	63	52.4	0.573	0.83	0.42 - 1.61
Neuter Status	0: Intact	72	62.5	-	1.0	-
	1: Neutered	68	47.1	0.068	0.53	0.27 - 1.05
*Age Quartile	0: ≤ 1.0 year	44	68.1	-	1.0	-
	1:1- 4years	66	43.9	0.014	0.37	0.16 – 0.81
	2: > 4 years	30	60.0	0.470	0.70	0.26 – 1.84
*Sex	0: Female	66	45.5	-	1.0	-
	1: Male	74	63.5	0.033	2.09	1.06 – 4.11
*Outdoor access	0: No	65	27.7	-	1.0	-
	1: Yes	75	78.7	0.007	2.59	1.30 – 5.17
Household	0: Single cat	31	51.6	-	1.0	-
	1: Multiple cat	109	56.0	0.668	1.19	0.54 – 2.65
Overall prevalence		140	55	-	-	0.47-0.63

Key: N = Number of cats; **P** = Probability value; **OR** = Odds Ratio; **CI =** Confidence Interval
***** These variables had Fisher's Exact P< 0.25 and were used in the multivariable logistic regression model.

Table 2. Multivariable logistic regression analysis of factors associated with *Toxoplasma gondii* seropositivity in domestic cats from selected areas around urban Harare (2017-2018)

Variable	Level	Multivariable logistic regression[d]				
		α	SE	P	OR	CI (95%)
	Constant	-1.96	0.19	0.050	0.40	0.16 – 1.00
Age Quartile	0: ≤ 1.0 year	-	-	-	1.0	-
	1:1- 4years	-1.15	0.29	0.25	0.53	0.18 – 1.56
	2: > 4 years	0.78	1.68	0.44	1.68	0.46 – 6.19
Sex code	0: Female	-	-	-	1.0	-
	1: Male	2.47	1.07	0.014	2.68	1.22 – 5.87
Outdoor Access	0: No	-	-	-	1.0	-
	1: Yes	3.84	3.0	0.000	6.21	2.44 – 15.79

Key: α = logistic regression coefficient; SE = standard error for the logistic regression coefficient; P = probability value; OR = Odds Ratio; CI = Confidence Interval.
Overall data for the model: Log likelihood = -82.28, LR Chi^2 (5d.f) = 28.13, P= 0.000, number of observations = 140.

Discussion

In the present study, a high overall sero-positivity of 55% was obtained, with the highest (78.7%) in cats with outdoor access and the lowest (27.7%) in those with no outdoor access. Variable prevalence rates for *T*. *gondii* antibodies in cats have been detected in similar studies in the region, depending on study areas, testing regimes and husbandry factors. In South Africa, IgG seroprevalences of 7.8%, 37.1% and 32.1% were obtained for urban Johannesburg cats with outdoor access, feral cats in Cape Town and cats from communities in south-east South Africa with free outdoor access, respectively (Lobetti and Lappin, 2012; Hammond-Aryee et al., 2015; Tagwirei et al., 2019). On the contrary, a low seroprevalence (3.9%) was reported in well cared for cats in Luanda, Angola (Lopes et al., 2017). The seropositivity recorded in the current study is also similar to those recorded from other countries outside the region: Svobodova et al., (1998) in the Czech Republic (61.3%), Maruyama et al. (2003) in Japan (63%), Meireles et al. (2004) in Brazil (40%) and Hornok et al. (2008) in Hungary (47.6%).

The difference in the seroprevalences among localities may depend upon aspects such as the density of outdoor cats, density of intermediate hosts and dietary habits of cats. Outdoor exposure was the greatest risk factor (OR: 6) for *T. gondii* seropositivity in this study. Outdoor access and hunting behavior are recognized as risk factors for *T*. *gondii* infection in cats (Opsteegh et al., 2012). Cats mostly get infected through ingestion of prey (Dubey, 2009).

Feeding raw meat donated by meat processors, as was the case at the shelters, could also contribute towards the cat infection. *Toxoplasma gondii* can also circulate among definitive hosts, without the involvement of the intermediate host, whereby a high dose of oocysts from the environment can be infective for other cats (Dubey, 2006). Low *T*. *gondii* seroprevalences have been linked to domesticated cats with no outdoor access and fed commercial, processed feed (Cerro et al., 2014).

The significantly higher seropositivity in males than females detected in the present work is similar to that observed elsewhere: USA (Vollaire et al., 2005), Christmas Island (Adams et al., 2008), and Iran (Javadi et al., 2010). Males tend to be more territorial and roam around larger areas in the environment, which increases their encounter with an infected intermediate host.

Household type in the current study did not show any significant association with seropositivity. This could have been due to the fact that most of the cats, whether from a single or multiple cat home, had outdoor access. Neuter status also had no significant association with seropositivity, maybe due to neutering later in life after exposure to *T. gondii* and the sampling strategy, including sample size and areas sampled, which might not have been a true representation of the population with respect to this particular factor. Timely neutering together with not feeding of raw meat to cats, proper disposal of cat litter and restricting hunting by domestic cats should be part of owners' education meant to contribute towards the reduction of acquired infections (Opsteegh et al., 2015).

Age showed a significant association with seropositivity. The high antibody titres in the first months of life could have partly been attributed to maternal antibodies (Opsteegh et al., 2012) which subsequently wane off at 3-4 months of age (Afonso et al., 2006). Exposure to natural infection after the waning of maternal antibodies may also have contributed to the high sero-conversion in the <1 year age group.

Even though the indirect ELISA test used has a high diagnostic sensitivity 99.6% (95% CL: 97.59 - 100%) and high specificity 100% (95% CL: 98.25 -100%), it only shows that the cat was exposed to the parasite at some point in its life (Montoya, 2002) but does not indicate whether the cat was shedding oocysts at the time of sampling. For 21 of the cats included in this study, faecal floatation did not detect any oocysts in both sero-negative and sero-positive groups. Sensitivity of light microscopy for oocyst detection is further reduced by the fact that only 1% of cats are found shedding oocysts at any point in time (Jones and Dubey, 2010). However, it has been demonstrated that cat immunity against oocyst shedding decreases over the years and this could trigger re-shedding of oocysts. Cats excreted millions of oocysts at first exposure to *T. gondii* (Dubey, 1995; Zulpo et al., 2017), had no oocyst-shedding on homologous strain challenge after 1-3 months (Davies and Dubey, 1995; Dubey, 1995) and showed increased oocyst-shedding on heterologous challenge after a number of years (Dubey, 1995; Zulpo et al., 2018). Concurrent infections with immunosuppressive pathogens such as the Feline Leukaemia virus (FeLV) and Feline Immunodeficiency virus (FIV) can also trigger the re-shedding of oocysts in older seropositive cats (Bresciani et al., 2016). Copro-antigen assays for the detection of *T. gondii* oocysts or their antigens, similar to those for protozoa such as *Giardia* and *Cryptosporidium*, could also improve diagnostic specificity and sensitivity as compared to the faecal flotation test (Behr et al., 1997). There are attempts to develop copro-PCR methods for detection of *T. gondii* oocyst nucleic acid (Salant et al., 2010) even though their reliability in certain circumstances has been questioned (Poulle et al., 2016).

Given the high seroprevalence of 55% recorded in this study, improved public awareness on *T. gondii* infection, the associated risk factors and available control measures which can minimise the infection levels in high risk groups is recommended. In particular, children, pregnant women and immunocompromised individuals should adhere to hygienic principles when handling soil and cats. Screening for antibody titres in high-risk groups such as pregnant women is also recommended.

Conclusion

This study revealed a high IgG seropositivity to *T. gondii* in cats from urban Harare. The highest seropositivity in those cats with free access to the outdoor further indicated that the activity was a major risk factor for acquisition of *T. gondii* infection.

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Conflict of interest

The authors declare that there were no conflicts of interest.

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