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Seroprevalence and molecular detection of *Toxoplasma gondii* infections in rodents and cats in Mbeya District, Tanzania

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Abstract

Toxoplasmosis is a zoonotic disease that is increasingly becoming an important but neglected disease of global concern. Cats and other feline species are the definitive host while rodents are the reservoirs of *Toxoplasma gondii* the causative agent of the disease. The study aimed to establish both Toxoplasma infection and exposure status in rodents and cats in Mbeya District, Tanzania. A cross-sectional study was employed where a total of 300 rodents and 52 cats were obtained from purposively selected wards in Mbeya district. To establish the infection statutes the serum and brain tissue in rodents and cats were collected for laboratory analysis. Indirect ELISA (ID.vet Innovative Diagnostics-France) was employed to determine *T. gondii* infection status in sera where seropositive samples and some of cat seronegative sample were subjected to conventional PCR analysis targeting GRA 6 gene for confirmation of the infection. Serological assay detected exposure status in 8.7 % (95% CI=0.057-0.124) of the rodent's serum samples and none in cats. PCR assay confirmed the infection in five of the rodent seropositive samples and one seronegative cat sample. Most of the seropositive samples belonged to *Rattus rattus* the most abundant (57%) rodent species captured in households and storage facilities. No significant variations in seroprevalence were found between locations, sex, species, or habitats. It is concluded that the study established the exposure status and actual infection of *T.gondii* in rodents in the study area and that *R. rattus* were the most exposed and infected. This highlights the risk of transmission of the infection to humans given the possible interaction with rodents in houses and stores where they were commonly trapped. The community health education on aspects of disease transmission, clinical signs and disease management is recommended. Furthermore, a regular animal and human screening for the disease to reveal its epidemiological patterns is also recommended.

Keywords: *cats; gene neglected diseases; GRA 6 genes; rodents, seroprevalence; toxoplasma spp; Zoonosis.*

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Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite infecting animals and humans. The parasite causes toxoplasmosis in many warm-blooded animal species worldwide (Murebwayire *et al.,* 2017; Nguemaim *et al.,* 2020). Toxoplasmosis has been recognized as a public health concern that places at least one third of the global human

population at risk of the infection (Graham *et al.,* 2021). Economic losses associated to this disease in the animal industry occur as a consequence of elevated animal mortality rates, abortions and treatment costs (Swai and Schoonman, 2009; Wang *et al.,* 2013; Hajj *et al.,* 2021). The burden of the disease is reflected by the prevalence of the pathogen in animals and humans located in different areas (Dubey *et al.,* 2012). *Toxoplasma gondii* exists in three forms namely; tachyzoites, bradyzoites and sporozoites. The forms occur as three consecutive and distinct stages in the parasite's life cycle (Wellington *et al.,* 2009). The tachyzoites are linked to asexual reproduction and tend to occur during acute stage of the infection unlike the bradyzoites which occur in the chronic stage. The sporozoites on the other hand are infectious forms formed during sexual reproduction of this parasite where they are ultimately released into the environment with potential to infect a new susceptible host (Borelli *et al.,* 2020).

Rodents are the most prevalent and diverse mammal species on the globe and are known to harbor a variety of zoonotic protozoan parasites (Wang *et al*., 2017; Onyuok *et al.,* 2019; Zhang *et al.,* 2022). These mammals reserve *T. gondii* microbes and serve as intermediate hosts in the pathogen's life cycle. Infected rodents contain encysted forms of the pathogen in different body tissues particularly in the muscle and brain. These tissues cysts can be consumed by their predators in the ecological web. Such predators may include feline pets that may subsequently pass the infection to other hosts via various means of pathogen shedding and disposal (Gryzbek *et al*., 2021). Rodents can also transmit the pathogen directly to humans in societies whose population consumes rodents' meat (Galeh *et al.,* 2020).

Cats play an important role in controlling rodents in homes, farms, store rooms and factories (Brown and Khamphoukeo, 2010; Krijger *et al.,* 2019). The cats get the infection through consuming rodents with *T.gondii* parasites (Krijger *et al.,* 2019; Hossein *et al.,* 2021). Domestic and wild cats are the only animal species known to shed environmentally resistant *T. gondii* oocysts in their feces. The capacity of cats to shed and discharge millions of oocysts with potential to infect other host species makes them principal hosts in the epidemiology of toxoplasmosis (Dubey *et al*., 2020). Control of pathogen shedding and spread from cats is the most feasible approach that could be employed in controlling transmission of the disease (Opsteegh *et al.,* 2015; Hossein e*t al.,* 2021).

In many places, humans co-exist with rodents and cats and share an ecological niche in a household setting. The important route of humans acquiring *T. gondii* infection is through exposure to contaminated soil, water or infected meat. These infectious substances contain either cysts or oocysts from either an infected intermediate or definitive host, respectively (Nguemaim *et al.,* 2019; Morais *et al*., 2021). Data reflecting the prevalence of this pathogen in rodents and cats in household settings is necessary to reveal the level of infection in the area and can provide an insight into the subsequent risk of disease transmission to humans.

Serological assays enable determination of active and passive infections however, such assays tend to estimate a value either lower or higher than the real value reflecting the prevalence of toxoplasmosis in an area (Mercier *et al*., 2013; Dubey *et al*., 2012; Galey*et al.,* 2020). Results from serological assays can be further subjected to molecular techniques which enable detection and identification of the particular pathogen to confirm the infection.

The highest seroprevalence of *T. gondii* infection (24%) in rodents has been reported in Africa (Galey *et al.,* 2020), followed by South America (18%) (Galey *et al.,* 2020) while Europe had the least (1%) seroprevalence (Galeh *et al.,* 2020). Variation in these levels could be attributed to variation in climatic condition, environmental hygiene, management system, as well as differences in sensitivity and specificity of the assays employed in detection(Gangneux and Darde, 2012). These levels highlight the significance of rodents in maintenance and transmission of the parasite. On the other hand, the documented seroprevalence of the parasite in domestic cats was 52%, 51% and 27% in Australia (Montazeri *et al.,* 2020), Africa (Nahavandi *et al.,* 2021) and Asia (Montazeri *et al.,* 2020) respectively.
In Tanzania, the prevalence of the infection in humans has been reported to range from 4% to 60 % in the past 30 years (Schooman *et al*., 2010; Mose *et al*., 2020; Onduru and Aboud, 2021).The disease claimed lives of 188 humans in Tanzania during a ten-year period from 2006-2015 where case mortality rate per 100,000

population was highest in the country's southern region (Mboera *et al*., 2019). This might be a gross underestimation as many cases could have passed undiagnosed given the neglected nature of the disease.

This study determined seroprevalence and actual infection of rodents and cats with *T. gondii* in Mbeya District, Tanzania**.** Establishment of the extent of exposure and infection of rodents and cats in this district is important for developing strategies, by policy makers, for preventing infections of pets, livestock and human.

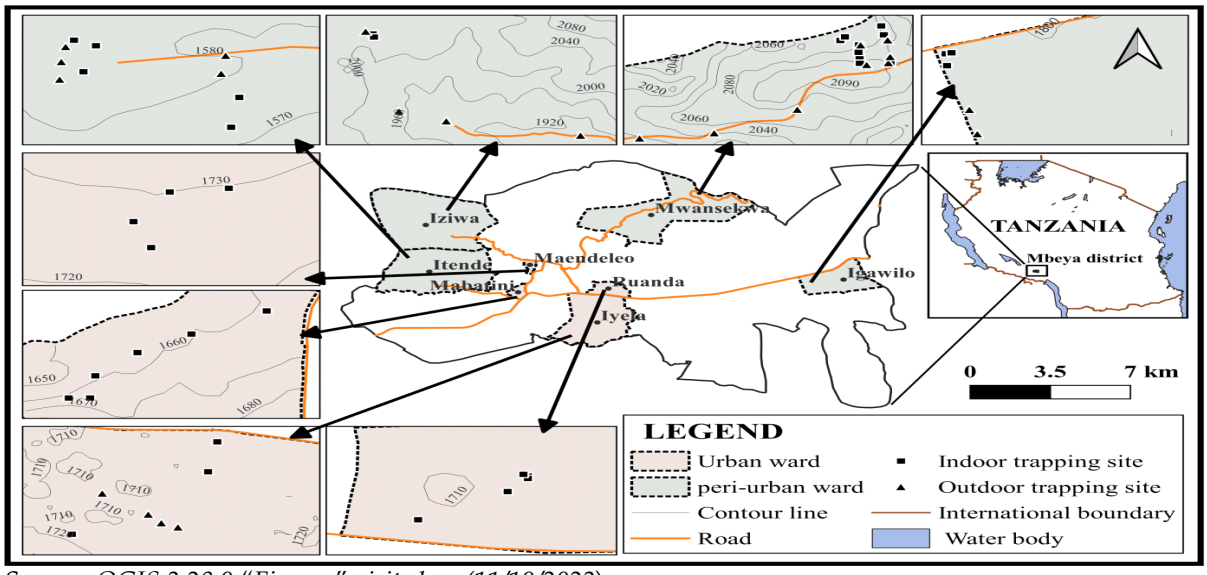
Materials and methods

Study Area

The study was conducted in urban and peri urban areas of Mbeya district in Mbeya region from January 2022 to March 2022. The district is located in the Southwest of Tanzania and lies along the crossings of latitude and longitude of $8^{0}54'$ south of Equator, 33^{0} 27'east of Greenwich Meridian respectively. The district is bordered by Mbarali, Ileje, Rungwe and Mbozi districts in the Northern, Southern, Eastern and Western, respectively (URT, 1997). Mbeya district's population is approximately 385,279 people (NBS, 2012). The district is administratively divided into 36 wards. The major economic activities in the district include business, agriculture and livestock keeping as well as small and large-scale industrial production (NBS, 2012). The climate is generally mildly warm and temperate. The annual average temperature is 7°C to 22 °C while the average rainfall is around 2068 mm per year (TMA, 2021). A total of eight wards (four periurban wards namely Igawilo, Mwansekwa, Itende, Iziwa and four urban wards namely Ruanda, Iyela, Mabatini and Maendeleo) were surveyed (Figure 1).

Figure 1

Map of Mbeya district showing surveyed wards



***Source**: QGIS 3.28.0 "Firenze" visited on (11/10/2022)*

Study Design and Sampling Strategies

A cross sectional study design was employed in this study. The wards were purposively selected based on zone characterization and their number evenly distributed between urban and peri urban zones. A total of eight wards (sampling sites) were selected. The target study population was rodents and cats. Sampling units considered in rodent trapping were human residences, grain storage facilities, forest areas, farm areas and fallow lands in the vicinity of human settlements. Sampling units considered in cat trapping were human residences and grain storage facilities.

Rodent trapping in household and storage facilities

Sampling units considered for rodent trapping were purposively selected within all study sites based on occurrence or complaints of rodent

infestation from respective residence owners. A total of 20 locally made live wire traps were used, whereby a maximum of two or three traps were set per night in each house involved in sampling. The number of traps set was based on the size of the house and information describing the presence and level of rodent infestation provided from household owners. Each trap was baited with tomatoes, bananas along with a mixture of peanut butter and maize bran. The traps were set at 1800 hours and inspected at 0700 hours at strategic points for three consecutive nights to increase capture rate (Mulungu *et al.,* 2008).

Rodents trapping in Farms, fallow lands and forest areas

A rodent trapping was conducted by using Sherman LFA live traps and Havahart traps with a mixture of peanut butter and maize bran, banana and tomatoes were used as baits (Mulungu *et al.,* 2008; Katakweba, 2018). 100 traps were set in each sampling site per night. An established grid of 100m x 100m containing a total of 10 lines each containing 10 traps was set 5 meters apart. This orientation was however allowed to vary in correspondence to the variation of the landscape in different habitats (Katakweba *et al.,* 2012). The traps were set at 1800 hours and inspected at 0700 hours for three consecutive nights to increase capture rate. The GPS coordinates of each capturing site were precisely recorded.

Cat trapping

Capture and release method was used to sample 52 cats using Havahart traps which were baited with fish to increase trapping efficiency then randomly placed inside the households and storage facilities. The GPS coordinates of the particular house hold or storage facilities where capturing was done were precisely recorded.

Rodents and cat identification

Three hundred rodents were captured and anesthetized by exposing cotton wool soaked with Di-Ethyl Ether on the rodent's nostrils. Anesthetized rodents were then identified based on their morphological features (body length, tail, fur, pes, and ear) according to morphological identification keys and illustrations developed by Meredith and Happold, (2013). Morphometric parameters including weight, sex, head body length, tail, left leg, and ear length measurements were then recorded. Cats on the other hand, were identified with aid of an international guideline developed by Bell *et al.,* (2012).

Blood and tissue sample collection

Blood and brain tissue samples were collected from 300 rodents while only blood samples were exclusively collected from 52 cats. Approximately, 3 millilitres of blood samples were collected from rodents by intra-cardiac puncture (Parasuraman *et al.,* 2010). Brain tissues of rodents were excised from the brain by dissecting the skull in sterile conditions. The brain tissues were then preserved in 70% ethanol at room temperature. Cats were anesthetized via intramuscular injection of a combination of xylazine, ketamine and atropine with a dosage of 2mg/kg, 5mg/kg, and 0.04mg/kg respectively. Blood samples were then collected via an intravenous puncture of the cephalic vein (Siqueira *et al.,* 2013). Whole blood samples collected from each rodent and cat were centrifuged at 5000rpm for 5 minutes at 22°C. The sera supernatant was extracted then stored at -20°C for future use (Rafique *et al.,* 2017; Lopes *et al.,* 2008).

Analysis of Toxoplasma-Antibodies by indirect ELISA

Serum samples were tested for anti-Toxoplasma IgG antibodies by indirect ELISA technique using an ELISA kit (ID.vet Innovative Diagnostics-France) following the manufacturer's instructions protocol. The kit included both positive and negative control sera. Toxoplasma IgG specific antibodies in diluted serum samples from both rodents and cats were allowed to bind to microwell-bound Toxoplasma antigens (p30) and incubated for 45 minutes at room temperature then washed three times with 300 µl of washing solution to remove unbound material. 100 µl of a Multispecies conjugate peroxidase (HRP) were then added and then the mixture was then incubated for 30 minutes for the enzyme to bind Toxoplasma IgGAb-Ag complexes. The wells were then properly emptied and washed by 300 µl of washing solution to remove the unbound conjugate. The substrate (Tetramethylbenzidine/TMB) was then allowed to react with bound peroxidase conjugate in each well. This peroxidase-TMB reaction was stopped by addition of a stop solution (0.5 M of Sulphuric acid). The optical density (OD) values for the indirect ELISA were determined at a wavelength 420nm by

using ELISA plate reader (ID.vet Innovative Diagnostics-France).

Sample to positive ratio value calculated using the following formula;

$$S/P\ \% = \frac{ODS-ODNC}{ODPC-ONDC} \times 100$$

Where; S/P% = Sample to positive ratio, ODS =Optical density of sample, ODNC=Optical density of negative control, ODPC= Optical density of positive control.

The criterion for deciding on positive or negative samples were done based on the obtained value from sample to positive ratio (a relative amount of antibodies in rodent and cat serum samples calculated by reference to the positive control) formula defined as S/P%≤40% was considered as negative, 40 %< S/P %< 50% Doubtful while S/P%≥ 50% considered to be positive (Can *et al.,* 2014).

DNA extraction: Total DNA was extracted from brain tissues of rodents as well as from blood of cats and rodents using the Quick-Dna™ Universal Kit (ZYMO Research) as described by Wang et al. (2018). Briefly, 0.025g of the homogenised brain-tissue suspended in equal volumes (95µl) of water and tissue buffer was digested with 10µl of proteinase K following incubation at 55^0C for 3 hours. The digested tissue was centrifuged at 120000xg for one minute and 200µl of supernatant was recovered in a clean 1.5mL-Eppendorf tube. To bind the extracted DNA, 400µl of genomic binding buffer was added to 200µl of the supernatant. The mixture was then added into a Zymo-spin™ IIC-XL column and centrifuged at 12000xg for 1 minute. Thereafter, the bound DNA was washed using a series of washing steps using wash buffers. The bound DNA was recovered from the columns using elution buffer. Extraction of DNA from blood was done as described above except that 20µl of proteinase K, 200µl each of biofluid and cell buffer, as well as 200µl of blood were added to the digestion mixture. Incubation was done at 55^0C for 10 minutes. The concentration and quality of the extracted DNA was measured by using NanoDrop™ spectrophotometer (Thermo Fisher scientific ™) and stored at -20°C pending further analysis.

Amplification of Toxoplasma gondii-DNA: The extracted DNAs were amplified by using a conventional PCR targeting the GRA6 gene of *T. gondii* as described by (Fazaeli *et al.,* 2000; Saki and Khademvatan, 2014). A forward primer, 5-GTAGCGTGCTTGTTGGCGAC-3, and reverse primer, 5-ACAAGACATAGAGTGCCCC-3 were used for the amplification (Saki and Khademvatan,2014). The PCR were done in a 25µl reaction mixtures containing 12.5µl of One Taq 2x master mix, 10µM of both forward and reverse GRA6 primers, 8.5µl of nuclease free water and 3µl of purified DNA. The PCR amplification involved an initial denaturation at 95°C for 5 minutes followed by 35 cycles each of denaturation at 95°C for 30 seconds, annealing at 59°C for 1 minutes, extension at 72°C for 30 seconds and a final extension at 72°C for 10 minutes. The PCR analysis was done using the Proflex PCR system. The amplicons were electrophoresed using 1.5% agarose gel, stained with ethidium bromide and then visualized using uv-transilluminator employing gel doc (Vilber Lourmat machine).

Data analysis

The surveyed qualitative and quantitative data were entered and coded in Microsoft Excel 2017 spread sheets. Descriptive and inferential statistics were computed with aid of the Statistical Product and service solution (SPSS) software version 25, created by IBM Corporation, Armonk, NY, USA in 2017. Descriptive statistics was computed to determine proportion while Inferential statistical was performed by using a chi-square test run at $p \leq 0.05$ to test the null hypothesis that there is no significant variation of seropositive Toxoplasmosis cases between locations, species, sexes and habitats.

Ethical clearance

Ethical clearance was obtained from the Institutional review board of the Sokoine University of Agriculture with reference number (SUA/DPRTC/R/186/20) issued on 1/12/2021. Furthermore, permission to conduct the study in the area was obtained from the Mbeya district administrative authorities which initially allowed the conduct of this research activity in all the respective study sites (wards) with reference number (MCC/R.50/1/VOL.XXV/207) issued on 27/01/2022.

Results

Rodent and Cat Species Composition in Selected Habitats

Sampling of rodents and cats in the study was conducted in five different types of habitats with exposure to anthropogenic activities. A total of 300 hundred rodents that belonged to eight species were captured. *Rattus rattus* was the most abundant (52%) species captured in all selected habitats. Furthermore, the species was noticeably most abundant particularly in households and storage facilities. The forest habitat displayed another noticeable abundance of *Praomys spp* and *Lophuromys spp.* On the other hand, *Felis catus* was the only cat species trapped and appeared to be most abundant in households (96.2%) and storage facilities (3.8%) unlike other habitats (Table 1).

Table 1

Rodents and cat species composition in selected habitats

TYPE	Genus/Spp	SITE/HABITAT House hold	Storage Facility	Farm area	Forest	Woodfarm	Total	% Composition
Rodents	*Rattus rattus*	101	70	0	0	0	171	57
	Lemniscomys striatus	0	0	11	0	0	11	3.7
	Mastomys natalensis	0	0	20	0	0	20	6.7
	Mus musculus	0	0	8	0	0	8	2.7
	Grammomys spp	0	0	6	0	0	6	2
	Praomys spp	0	0	0	47	0	47	15.7
	Lophuromys spp	0	0	0	26	0	26	8.7
	Graphiulus spp	0	0	0	0	11	11	3.7
	Total for rodents	101	70	45	73	11	300	
Cats	*Total Felis catus*	50	2	0	0	0	52	100

Seroprevalence of Toxoplasmosis in rodents and cat

A total of 352 serum samples, were collected from 300 rodents and 52 cats and then subjected to serological assays to reveal the exposure status to *T. gondii* infection**.** The overall seroprevalence of Toxoplasmosis in all rodents captured in the study was 8.7 % (95% CI=0.057-0.124). Seropositive samples were detected in five among the eight different rodent species captured. *Rattus rattus* was the species with highest number (18) of seropositive samples. The remaining four seropositive species had less than five seropositive samples. On the other hand, seropositivity was not detected in any of the 52 cat serum samples assayed (Table 2).

Table 2

Seroprevalence of Toxoplasmosis among captured Rodents and Cat species

Type	**Genus/spp**	**Disease status**			**Seroprevalence (%)**
		Number tested	**Number seronegative**	**Number seropositive**	
Rodents	*Rattus rattus*	171	153	18	10.5
	Lemniscomys striatus	11	11	0	0
	Mastomys natalensis	20	19	1	5
	Mus musculus	8	8	0	0
	*Grammomys s*pp	6	6	0	0

	Praomys spp	47	43	4	8.5
	Lophuromys spp	26	25	1	3.8
	Graphiulus spp	11	9	2	18.2
	Total for rodents	300	274	26	8.7
Cats	*Total for Felis catus*	52	0	0	0

Statistical significance in seropositive cases within categorical variables

The Chi-square and Fisher-exact test were employed to determine association between the variables. The results revealed that the variation in the number of Toxoplasmosis seropositive cases within all selected variables lacked statistical significance (Table 3).

PCR results of GRA 6 gene of T. gondii

DNA detection was performed in 40 rodents' brain tissues and 10 cats' blood samples by subjecting them to conventional PCR to amplify the GRA 6 gene specific for *T. gondii*. 26 out of these were seropositive while the remaining 24 were negative. A total of six samples (5 isolated from rodents and one from cat gave positive results for GRA 6 gene molecular detection as (Figure 2).

Figure 2

PCR amplification of Toxoplasma gondii gene (TG). M is a 100bp marker and lane 1-5, 8-10 are samples, where lane 1-5 and 10 are positives at an expected band size of 790bp while lane 6 and 7 are negative and positive controls respectively.

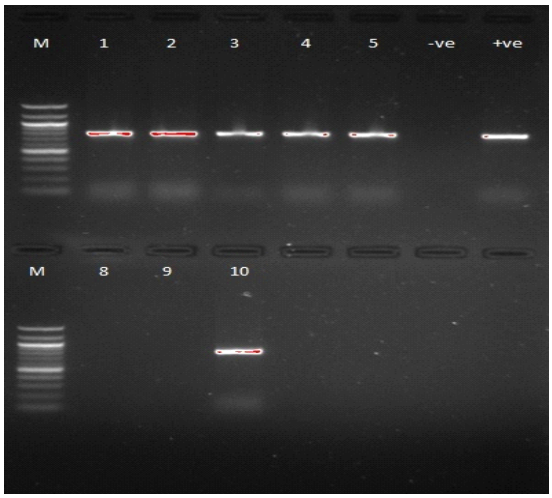


Table 3

Statistical inference of variation in seropositive cases within selected categorical variable

		Disease status				
Variable		**Seronegative**	**Seropositive**	**Total**	**X^2**	**P-value**
Location	Periurban	140(46.7%)	8(2.7%)	148(49.3%)	3.925	0.624
	Urban	134(44.7%)	18(6%)	152(50.7%)		
Species	*Rattus rattus*	153(51%)	18(6%)	171(57%)	5.482	0.581
	Lemniscomys striatus	11(3.7%)	0(0.0%	11(3.7%)		
	Mastomys natalensis	19(6.3%)	1(0.3%)	20(6.7%)		
	Mus musculus	8(2.7%)	0(0.0%)	8(2.7%)		
	Grammomys spp	6(2.0%)	0(0.0%)	6(2.0%)		
	Praomys spp	43(4.3%)	4(1.3%)	47(15.7%)		
	Lophuromys spp	25(8.3%	1(0.3%)	26(8.7%		
	Graphiulus spp	9(3.0%)	2(0.7%)	11(3.7%		
Sex	M	139(46.3%)	12(4.0%)	151(50.3%)	0.199	0.656
	F	135(45.0%)	14(4.7%)	149(49.7%)		

Site/habitat	Household	87(29.0%)	14(4.7%)	101(33.7%)	8.138	0.082
	Storage facility	66(22.0%)	4(1.3%)	70(23.3%)		
	Farm area	44(14.7%)	1(0.30%)	45(15.0%)		
	Forest	68(22.7%)	5(1.7%)	73(24.3%)		
	Woodfarm	9(3.0%)	2(0.7%)	11(3.7%)		

Discussion

This is the first study to elucidate the seroprevalence of *T. gondii* in rodents and cats in southern highlands in Tanzania. The findings reveal a seroprevalence of 8.7% in the captured rodents in Mbeya district. This implies a high risk transmitting the infection to humans due to the possibility of interaction in the shared environment. Rodents usually enter the houses for food and or shelter. Apart from the destructive effects the rodents cause also transmit diseases. Toxoplasmosis is one of the rare and neglected diseases transmitted by rodents causing chronic infection in infected individuals. The reported lack of community awareness about Toxoplasmosis in the study area increases the chances of contracting the infection (Chalo *et al.,* 2023).

The infection is wide spread, although at varying proportions. For instance, (Bahadori *et al*., 2019; Ahmad *et al*., 2012; Gryzbek *et al*., 2021) in Iran, Pakistan and Poland respectively reported higher seroprevalence while Mgode *et al*., (2014), Defeo *et al*., (2002) and Brouat *et al*., (2018) in Morogoro, Rhode Island and Senegal respectively registered lower seroprevalence rates. The observed variations in seroprevalence rates in different areas could be a function of variations in climatic and ecological properties such as humidity, soil topography, ambient temperature and exposure to ultraviolet rays in the studied areas. These factors are known to influence the viability of pathogenic *T. gondii* oocysts shed in the environment. Similar observations have been made by Dabritz *et al*., (2008) and Mercier *et al*., (2013). The Sporulated oocysts can remain viable for approximately 12 to 18 months in moist environments (Dubey, 2011; Yan *et al*., 2016). Increased precipitation furthermore accelerates the sporulation of *T. gondii* oocysts (Meerburg and Kijlstra, 2009). Mbeya Districtis characterized by climatic conditions favoring survival and sporulation of the oocysts where the climate is mildly warm and temperate according to the data provided by the Tanzania Meteorological Authority (TMA, 2021). The *T.gondii* oocysts contaminating the soil, vegetation or water remains infective for long periods in such climatic conditions and may subsequently be contracted by rodents seeking shelter or food (Jittapalapong *et al*., 2011). In addition, epidemiological studies on Toxoplasmosis have showed that the affected rodents are associated with behavior changes which make them more susceptible to cat predation (Mikhail *et al*., 2017). This increased rate of predation of infected rodents consequently reduces their numbers in a given area at a given time. This could be one of the factors influencing the variation in serological results. This factor was similarly reported by Defeo *et al*., (2002) and Mikhail *et al*., (2017) in Rhode Island and Egypt respectively. Furthermore, the variation in serological results from various studies could be partly attributed to differences in sensitivity and specificity of the serological assays employed in detection (Defeo *et al*., 2002; Galeh *et al*., 2020).

The seropositive samples belonged to the *Rattus rattus* the most abundant (57%) rodent species captured in the study area reflecting high infection rates in these habitats. The higher seroprevalence in the species could be associated with their higher tendency to co-exist with humans as a commensal rodent species hence amplifying their risk of acquiring infections from media contaminated by oocysts shed by domesticated cats(Byers *et al*.,2019;Gotteland *et al*.,2013). Given the close interaction of *R. rattus* with humans, the detected *T. gondii* infection in these rodents poses a high risk of transmission to humans.

Lack of seropositivity in other three rodent species such as *Lemniscomys striatus*, *Mus musculus* and *Grammomys spp* does not means absence of the infection. It could be attributed to the low number of the rodent species sampled, therefore is not sufficient to exclude them from involvement in transmission of Toxoplasmosis. Similar observation was reported by Jittapalapong *et al*., (2011).

Based on the statistical inference, results revealed that variation in the number of

seropositive cases within selected categorical variables lacked statistical significance as similarly observed by Krijger *et al.,* (2019). However, this result appears contrary to that obtained in other studies which revealed that the variation in rodent infections within species, geographic location, and season was statistically significant (Gotteland *et al.,* 2014; Morand *et al.,* 2015). The absence of a significant relationship between the seroprevalence of Toxoplasma infection among rodents inhabiting Mbeya implies that all variables (location, sex, species and habitat) face equal risk of being infected with *T. gondii* infection.

On the other hand, *T. gondii* infection was not detected in cats using serological assays contrary to other reported studies which detected the infection (Can *et al.,* 2014; Lopes *et al.,* 2008; Sioutas *et al.,* 2022). The variation in such results could be a result of several reasons including the amplitude and strength of the humoral response in the particular hosts among other factors (Jokelainen *et al.,* 2012). The seronegative samples could have been drawn from cats whose humoral response to *T. gondii* had low strength and amplitude hence difficult to detect. Cat management status also influences the seropositivity, the stray cats are at higher risk of acquiring the infection than domesticated ones. All the cats sampled in this study were domesticated probably influencing the observed dominant seronegativity of their samples. This supports the results of studies conducted in Netherland and Greece (Opsteegh *et al.,* 2012; Sioutas *et al.,* 2022). Furthermore, according to Sioutas *et al.,* (2022) other factors that could influence the variation in seropositivity of such results include; variation in sensitivity of the serological assay applied in the studies, sampling technique employed where the number of positive cat samples could have been reduced by the bias introduced by purposive selection.

Serological based assays have some shortfalls. They are of low sensitivity, less specificity and detect both active and passive infections. Molecular assays are used to confirm the active infection given their high sensitivity and species specificity. Molecular detection of GRA 6 gene in some of the seropositive rodents and seronegative cats confirms the *T. gondii* infection. The results reflecting fewer molecular confirmations of *T. gondii* infections in seropositive rodents and cat samples are in line with those obtained in study conducted in Iran (Bahadori *et al.,* 2019).

Variation of this results could be attributed to serological cross-reactivity which may occur between antigens sharing similar epitopes with the *T. gondii* antigen (Nishikawa *et al.,* 2002). This has been similarly claimed by Gondim *et al.,* (2017) in Cambridge who reported that there is cross reactivity among cyst forming coccidian parasites including *T. gondii, Hammondia* spp, *Neospora* spp, *Sarcocystis* spp, and *Besnoitia besnoiti*. The molecular results could have also been affected by the stage of parasites life cycle as the GRA 6 gene is more abundant in the bradyzoites unlike tachyzoites stage (Bahadori *et al.,* 2019). Additionally, in previous infection where parasites have been cleared but antibodies persist for longer periods may also influence the relatively lower molecular detection since the study was detecting immunoglobulins (IgG's) which persist longer in the body and are produced in the delayed response (Elmore *et al.,* 2010). Presence of doubtful sample to be positive in PCR supports features of PCR of being characterized as a highly sensitive and specific molecular technique in detection the parasite in the living organisms (Saki and Khademvatan, 2014).This implies that some serological assay may underestimate the infection status of a pathogen due to high threshold level set as also reported in France (Gotteland *et al.,* 2014).

Conclusion

It is concluded that the study established the exposure status and actual infection of *T. gondii* in rodents in the study area and that *R. rattus* were the most exposed and infected. This highlights the risk of transmission of the infection to humans given the possible interaction with rodents in houses and stores where they were commonly trapped. To prevent disease transmission to humans, eco-friendly approaches to control rodents and cats are recommended. Furthermore, continuous public health education and advocacy as well as regular screening of *T. gondii* infection in both humans and animals should be considered. Also further molecular analysis is recommended to reveal genotypic details of the pathogen and could be useful in revealing molecular epidemiological patterns of the disease.

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