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Detection of *Toxoplasma gondii* Infection in Rodents, Shrews and Cats in Unguja Island, Zanzibar

Deteksi Infeksi *Toxoplasma gondii* Pada Rodensia, Curut dan Kucing di Pulau Unguja, Zanzibar

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ABSTRACT

Background: *Toxoplasma gondii* is an intracellular protozoan parasite that causes a zoonotic infection known as toxoplasmosis. Felid vertebrates including cats serve as the definitive hosts of *T. gondii* and rodents and shrews are among the common intermediate and reservoir hosts. Little is known about the infection in both humans and animals despite the high interaction between humans and cats on the island. **Purpose:** This study aimed at determining the prevalence of *T. gondii* infection in rodents, shrews and cats in Unguja Island, Zanzibar. **Method:** The study employed a cross-sectional design. A total of 366 small mammals (230 rodents, 43 shrews and 93 cats) were captured and blood was sampled from the seven districts of Unguja Island. Serum samples were subjected to the Indirect ELISA technique with the aid of the ID Screen® Toxoplasmosis Indirect Multi-species Kit to look for antibodies directed against *T. gondii*. **Results:** Seropositive samples were detected from one rodent species *(Rattus rattus)* and one shrew species *(Crocidura spp.)* among the six different rodent and shrew species captured. Fifty-one seropositive samples from cats were detected where eight were from pet cats and 43 from stray cats. In rodents, no significant variation was found according to species, sex and habitat ($p \geq 0.05$) but in cats, there was a significant variation between habitats ($p \leq 0.05$). **Conclusion:** The study revealed the exposure status of rodents, shrews and both stray and pet cats in Unguja Island, thereby revealing the potential risk of the infection transmission to humans due to close proximity with these animals, especially cats as pets. Emphasis should be made through public health education programs about the transmission of the infection and the control measures needed to mitigate the risks.

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ABSTRAK

Latar Belakang: *Toxoplasma gondii* adalah parasit protozoa intraseluler yang menyebabkan infeksi zoonosis yang dikenal sebagai toksoplasmosis. Vertebrata felid termasuk kucing berfungsi sebagai inang definitif *T. gondii* dan hewan pengerat serta tikus tanah termasuk di antara inang perantara dan reservoir yang umum. Sedikit yang diketahui tentang infeksi pada manusia dan hewan meskipun interaksi antara manusia dan kucing di pulau tersebut tinggi. **Tujuan:** Penelitian ini bertujuan untuk menentukan prevalensi infeksi *T. gondii* pada hewan pengerat, curut, dan kucing di Pulau Unguja, Zanzibar. **Metode:** Penelitian ini menggunakan desain cross-sectional. Sebanyak 366 mamalia kecil (230 hewan pengerat, 43 curut, dan 93 kucing) ditangkap dan diambil sampel darahnya dari tujuh distrik di Pulau Unguja. Sampel serum dikenakan teknik ELISA Non Direct dengan bantuan ID Screen® Toxoplasmosis Indirect Multi-species Kit untuk antibodi terhadap deteksi *T. gondii*. **Hasil:** Sampel seropositif terdeteksi dari satu spesies hewan pengerat (Rattus rattus) dan satu spesies tikus tanah *(Crocidura spp.)* di antara enam spesies hewan pengerat dan tikus tanah yang ditangkap. Lima puluh satu sampel seropositif dari kucing terdeteksi, delapan dari kucing peliharaan dan 43 dari kucing liar. Pada hewan pengerat tidak ditemukan variasi signifikan dalam spesies, jenis kelamin, dan habitat ($p \geq 0{,}05$), tetapi pada kucing, terdapat variasi signifikan antar habitat ($p \leq 0{,}05$). **Kesimpulan:** Penelitian ini mengungkap status paparan hewan pengerat, tikus tanah, dan kucing liar maupun peliharaan di Pulau Unguja sehingga mengungkap potensi risiko penularan infeksi ke manusia karena kedekatan dengan hewan-hewan ini, terutama kucing sebagai hewan peliharaan. Oleh karena itu, penekanan harus diberikan pada program pendidikan kesehatan masyarakat tentang penularan infeksi dan langkah-langkah pengendalian untuk mengurangi risiko.

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Kata kunci: ELISA; Hewan Pengerat; Kucing; Protozoa; Seroprevalensi; Zoonosis

INTRODUCTION

Toxoplasma gondii is an intracellular apicomplexan protozoan parasite causing Toxoplasmosis in humans and animals worldwide (Dubey and Beattie, 1988). Its lifecycle is complex, with humans and other warm-blooded animals serving as intermediate hosts, and domestic and wild cats serving as final hosts (Elmore *et al.,* 2010). Cats are the primary source of infection for both humans and other animals because they release oocysts into the environment, which can live for up to 18 months depending on the environmental conditions (Innes, 2010).

Rodents and shrews are among the intermediate and reservoir hosts of *T. gondii* with a close interaction with cats as their predator. The infection in cats takes place after ingesting the tissue cysts in the intermediate hosts (Dubey *et al.,* 2009). Among the cat populations, Toxoplasmosis can spread through the fecal-oral transmission route by ingesting oocysts directly from contaminated environments or indirectly from contaminated food or water (Dubey, 1995). Infection in cats has no noticeable clinical signs unless the cats are immunocompromised or kittens (Vollaire *et al.,* 2005). The clinical signs associated with feline toxoplasmosis include fever, anorexia, abdominal discomfort, ocular inflammation, icterus, hyperpnea, lethargy, dyspnoea and CNS disturbances (Dubey and Beattie, 1988; Dubey and Carpenter, 1993). Because they are more likely to come into touch with oocyst-contaminated regions than indoor domestic cats, stray cats and outdoor cats are more likely to become infected.

Since cats and rodents share an environment with people, they play a significant role in the epidemiology of Toxoplasmosis. In humans, the two major sources of infection are through consuming oocyst contaminated food and water, and the ingestion of undercooked meat that has T. gondii tissue cysts (Dubey, 2021). According to Jilo *et al.,* (2021), the infection is typically asymptomatic in immunocompetent individuals and more dangerous in immunocompromised people and pregnant women. The clinical signs shown by these groups include abortion, epilepsy, encephalitis, schizophrenia, ocular disease and lymphadenopathy (Montoya and Liesenfeld, 2004; Weiss and Dubey, 2009).

T. gondii infections affect between 25 and 30% of people on the planet (Montoya, 2002). In Africa, this prevalence varies widely across different regions and populations. Studies have shown ranging rates of infection across the continent, with estimates ranging from 10% to 80% in different regions (Torgerson and Mastroiacovo, 2013). Within East Africa, Tanzania has varying rates of Toxoplasmosis prevalence. Research has shown that Toxoplasmosis prevalence in Tanzania ranges from 4% to 60% (Onduru *et al.,* 2019), including a study conducted in the Tanga district whereby 46% of individuals studied tested positive for *T. gondii* (Swai and Schoonman, 2010). A study conducted in Mwanza found that about 30% of pregnant women tested positive for *T. gondii*-specific antibodies (Mwambe *et al.*, 2013).

Africa (24%) and South America (18%) have the highest serological prevalence of *T. gondii* infections in rodents, whereas Europe (1%), had the lowest seroprevalence (Galeh *et al.,* 2020). In cats however, the global seroprevalence of *T. gondii* has been estimated to be 35% (95% CI: 32–38%) in domestic cats and 59% (95% CI: 56–63%) in wild cats, respectively (Montazeri *et al.,* 2020). However, not much is known regarding the prevalence of *T. gondii* in rodents and cats in Zanzibar, Tanzania. With an aim of gathering information for developing preventative measures against the infection in humans and animals, this study examines the prevalence of *T. gondii* infection in cats, rodents and shrews.

MATERIALS and METHODS

Study Area

The study was conducted on Unguja Island, Zanzibar from February to April, 2023. Unguja is an Island located off the eastern coast of Tanzania (6° 08′ 26.00" S, 39° 20′ 11.57" E), with a land area of 1,666 Km2 and a population of 1,346,332 people (NBS, 2022). It is characterized by an equatorial and humid climate with relatively stable temperatures year-round, averaging between 25°C to 30°C, with high humidity levels often exceeding 80%. People living in Unguja depend on several sources of income including activities based on tourism, agriculture, animal husbandry, seaweed farming and fishing. Unguja Island has three regions, Kaskazini Unguja, Kusini Unguja and Mjini Magharibi, with seven districts included in this study, specifically Magharibi A, Magharibi B, Kaskazini A, Kaskazini B, Mjini, Kati and Kusini (Figure 1).

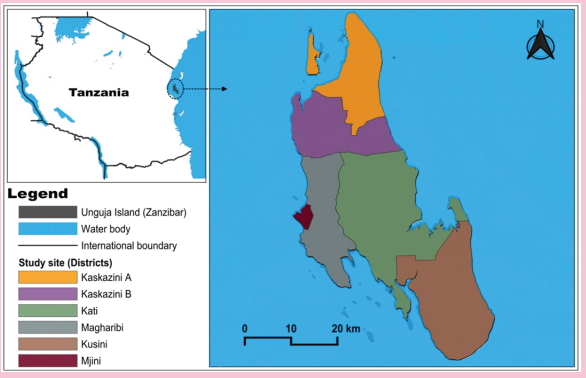

Figure 1. Map of Unguja Island showing the surveyed districts (Qgis 3.34.1 – Prizren; EPSG: 4326 – WGS 84)

Study Design and Sampling Strategies

A cross-sectional study design was adopted, whereby serum samples were taken from rodents, shrews and cats. A total of 28 shehia were included through random selection, four from each district. The selected shehia in each district included Magharibi A (Dole, Masingini, Kianga and Mtoni), Magharibi B (Mombasa, Mwanakwerekwe, Kiembe samaki and Nyamanzi), Kati (Binguni, Tunduni, Chwaka and Mchangani), Mjini (Jang'ombe, Kikwajuni, Malindi and Kiponda), Kaskazini A (Bandamaji, Kinyasini, Potoa and Kikobweni), Kaskazini B (Fujoni, Mangapwani, Mkataleni, and Mahonda), and Kusini (Bwejuu, Paje, Kajengwa and Nganani).

Inclusion and Exclusion Criteria

For rodents and shrews, the study included live juveniles, sub-adults, and adults (Mills and Childs, 1998). For cats, the study included senior cats (11-14 yrs), adult cats (3-6 yrs), junior cats (1-2 yrs) and kittens older than 4 months (Bellows *et al.,* 2016; Hoyumpa Vogt *et al.,* 2010). Kittens below 4 months were not included due to their small size as it was not possible to collect enough blood from them. Likewise, cat owners who were not ready to participate in the study were not included.

Rodents and Shrews Trapping

Rodents and shrews were captured in and around human dwellings and farms proximal to human settlement such as cultivated, fallow lands and grazing pasture lands using sherman live traps and locally-made live traps. The bait used was either avocado, ripen banana, green maize or a mixture of peanut butter and maize bran (Mulungu *et al.,* 2008). The traps were placed in the evening and collected the following morning before 09:00 am. All captured animals were transported to the laboratory of the Department of Veterinary Development in Zanzibar for blood sample collection and identification using taxonomic keys according to Happold *et al.,* (2013).

Blood Collection from Rodents and Shrews

The captured rodents and shrews were anaesthetized using diethyl ether-soaked cotton wool prior to identification. External morphological measurements including body weight, body and head length, as well as tail, ear and hind foot length, were recorded for primary identification. Blood samples were collected through heart puncture using 2ml sterile syringes and needles (McClure, 1999; Parasuraman *et al.,* 2010). Afterwards, the blood was put into plain Eppendorf tubes, and was centrifuged for 10 - 15 minutes at 1300 to 2000 rpm to get clear serum. Serum separation occurred at a room temperature for 30 minutes after the tubes were allowed to clot. The serum was then transferred into plain Eppendorf tubes, which were kept frozen at -20°C in the Department of Veterinary Development laboratory in Zanzibar. These tubes were later shipped in cool boxes to the College of Veterinary Medicine and Biomedical Sciences Research Laboratory in the Sokoine University of Agriculture. Before the ELISA analysis, the serum samples were frozen at -20°C.

Cat Trapping

The capture and release method was used to capture the cats where havaharts traps baited with either fish or sardines were used to trap cats in households, slaughter slabs, veterinary centers, dumping sites and hotels (Short *et al.,* 2002). The traps were set during the evening hours and taken to the cats' clinic for blood collection the following morning.

Blood Collection from Cats

Before blood sampling, the cats were anaesthetized using a combination of ketamine, atropine and xylazine at a dosage of 5mg/kg, 0.04mg/kg and 2mg/kg, respectively via intramuscular injection (Fleming *et al.,* 2021). The cats' weight was measured and their age determined through the observation of their teeth (Feldman and Nelson, 2004). Then, their blood was collected through venipuncture from the lateral tail, jugular or cephalic vein using 2ml sterile syringes and transferred into plain vacutainer tubes (Zhang *et al.,* 2018). The samples were transported to the laboratory where they were centrifuged for 10 to 15 minutes at 1300 to 2000 rpm to obtain a clear serum sample. The serum was then put into plain Eppendorf tubes which were stored frozen at -20°C, until they were transported to the College of Veterinary Medicine and Biomedical Sciences Research Laboratory at Sokoine University of Agriculture in cool boxes. They were storing frozen at -20°C until used for the ELISA. Pet cats were returned to their owners while the stray cats were returned to where they were taken in cages after marking them to avoid recapture.

Serology Technique

ID Screen® Toxoplasmosis Indirect Multi-species Kit was used as an Indirect Enzyme-Linked immunosorbent assay technique in order to detect antibodies directed against *T. gondii* for all serum samples from cats, rodents and shrews. All of the manufacturers' instructions were observed during the test procedures. Optical Density (OD) was measured at 450 nm using an ELISA plate analyzer (MICRO READ 1000, Inqaba biotech™). As per the manufacturer's protocol, the results were considered valid if the ratio of the mean O.D. values of the positive and negative control was more than 3, and the mean value of the positive control O.D. was greater than 0.350. The value of the sample to positive ratio was determined using the following formula:

$$S/P\ \% = (OD_{Sample} - OD_{NC} / OD_{PC} - OD_{NC}) \text{ X } 100$$

Where, S/P% is the Sample to positive ratio, OD Sample is the Optical density of the sample, ODNC is the Optical density of the negative control and ODPC is the Optical density of the positive control.

Data Analysis

Spreadsheets made with Microsoft Excel 2019 were used to enter and code the collected data. The Statistical Product and Service Solutions (SPSS) software, formerly known as Statistical Package for Social Sciences version 25, which was developed in 2017 by IBM Corporation, was utilized to compute both the inferential and descriptive statistics (George and Mallery, 2018). Descriptive statistics were used to determine proportion while the results of the inferential statistical analysis using a chi-square test were considered statistically significant at $p \leq 0.05$.

RESULT

Rodents, Shrews and Cats Captured

A total of 273 rodents and 43 shrews from domestic, peridomestic, grazing, farm, and forest areas were sampled. Out of these, 128 were male and 145 were female. In the peridomestic habitats, *R. norvegicus* was the most common species, while *Rattus rattus* was the most common species in the domestic habitats and *M. natalensis* was the most common species on farms. A total of 93 cats were sampled from peridomestic and

domestic areas including stray cats and client-owned cats respectively. Out of 93 cats captured, 30 were male and 63 were female (Table 1).

Seroprevalence of Toxoplasmosis in Rodents, Shrews, and Cats

The overall seroprevalence of Toxoplasmosis in rodents and shrews was 0.74%. Seropositive samples were detected from one rodent species and one shrew species. *Rattus rattus* was the rodent species with one seropositive sample and *Crocidura spp.* was the shrew species with one seropositive sample. The overall seroprevalence of Toxoplasmosis in cats was 54.84%. Stray cats showed the highest number of seropositive results compared to client-owned cats (Table 2).

Statistical Significance in Seropositive Cases within Categorical Variables

To determine the association between the variables, the chi-square test was employed. The seropositivity of toxoplasmosis in rodent and shrew variations in all selected categorical variables showed no statistical significance ($p \geq 0.05$) (Table 3). However, in cats, habitat showed there to be a statistically significant association between the number of Toxoplasmosis seropositive cases and habitat ($p \leq 0.05$). Other variables lacked statistical significance ($p \geq 0.05$) (Table 4).

DISCUSSION

Sequencing Analysis

In Unguja Island, Zanzibar, this study determined the presence *T. gondii* infection in rodents, shrews and cats. In this study, which is the first on the Island, the overall seroprevalence of Toxoplasmosis was found to be 0.74% in rodents and shrews, and 54.84% in cats. This suggests a significant risk to public health. A similar study was conducted in Mbeya, whereby the overall seroprevalence of Toxoplasmosis obtained in rodents was 8.7 %. However, seropositivity was not detected in any of the cat serum samples assayed (Chalo *et al.,* 2023). This difference in Toxoplasmosis seroprevalence may be influenced by climatic factors, environmental conditions, density and behavior of the hosts, sampling season, sample size, and geographic location.

The overall *T. gondii* seroprevalence in cats in the current study was higher (54.84%) compared to rodents and shrews (0.74%). This could be a result of cats being the definitive hosts for *T. gondii,* meaning that the parasite completes its life cycle in the feline intestines while rodents have a shorter lifespan and different exposure patterns. Studies conducted in Niger and Senegal also revealed a low prevalence of *T. gondii* infection rate in rodents of about 1.96% and 4%, respectively (Mercier *et al.,* 2013; Brouat *et al.,* 2018). Another study conducted in Morogoro, Tanzania revealed a similar finding, whereby *T. gondii* was detected in one rodent out of 90 small mammals captured (Mgode *et al.,* 2014). The variations of these prevalence rates may be due to the environmental conditions that determine shelter and food availability, which affects the distribution and abundance of rodents and their predators (Meerburg *et al.,* 2009). Climatic factors can also influence the occurrence of *T. gondii* infection in cats and their prey because oocyst survival depends on climatic and physical conditions (Afonso *et al.,* 2010).

Stray cats have greater exposure to outdoor environments where they may come into contact with infected animals, contaminated soil, and other sources of the parasite. This explains the higher prevalence of *T. gondii* observed in stray cats (46.24%) compared to client-owned cats (8.60%) in the

Table 1. Rodent, Shrew and Cat Species Captured in Unguja Island.

Type	Species/Genus	Habitat	Male	Female	Total	%
Rodents	*Mus spp.*	Domestic	16	17	33	12.09
	Rattus rattus	Domestic/Grazing	67	62	129	47.25
	Cricetomys spp.	Domestic/Peridomestic	9	6	15	5.49
	Rattus novergicus	Peridomestic	14	19	33	12.09
	Mastomys natalensis	Farm	7	13	20	7.33
Shrews	*Crocidura spp.*	Forest	15	28	43	15.75
	Total Rodents and Shrews		**128**	**145**	**273**	**100.00**
Cat	*Felis silvestris catus*	Domestic/Peridomestic	**30**	**63**	**93**	**100.00**

Table 2. Seroprevalence of *T. gondii* in Rodents, Shrews and Cats Captured in Unguja Island.

		Tested	Seropositive	Seronegative	Seroprevalence %
Rodents	*Mus spp*	33	0	33	0.00%
	Rattus rattus	129	1	128	0.37%
	Cricetomys spp.	15	0	15	0.00%
	Rattus novergicus	33	0	33	0.00%
	Mastomys natalensis	20	0	20	0.00%
Shrews	*Crocidura spp.*	43	1	42	0.37%
	Total Rodents and Shrews	**273**	**2**	**271**	**0.74%**
Cat	Client-owned	33	8	25	8.60%
	Stray	60	43	17	46.24%
	Total Cats	**93**	**51**	**42**	**54.84%**

Table 3. Statistical Significance in Seropositive Cases within Selected Categorical Variable in Rodents and Shrews (Total % of 273).

Variables		Infection Status			p-value
		Seropositive	Seronegative	Total	
Species/genus	*Mus spp*	0 (0.00%)	33 (12.09%)	33 (12.09%)	0.814
	Rattus rattus	1 (0.37%)	128 (46.89%)	129 (47.25%)	
	Cricetomys spp.	0 (0.00%)	15 (5.49%)	15 (5.49%)	
	Rattus novergicus	0 (0.00%)	33 (12.09%)	33 (12.09%)	
	Mastomys natalensis	0 (0.00%)	20 (7.33%)	20 (7.33%)	
	Crocidura spp.	1 (0.37%)	42 (15.38%)	43 (15.75%)	
Sex	Male	2 (0.73%)	126 (46.15%)	128 (46.89%)	0.131
	Female	0 (0.00%)	145 (53.11%)	145 (53.11%)	
Habitat	Domestic	1 (0.37%)	91 (33.33%)	92 (33.70%)	0.613
	Peridomestic	0 (0.00%)	43 (15.75%)	43 (15.75%)	
	Grazing	0 (0.00%)	75 (27.47%)	75 (27.47%)	
	Farm	0 (0.00%)	20 (7.33%)	20 (7.33%)	
	Forest	1 (0.37%)	42 (15.38%)	43 (15.75%)	

Table 4. Statistical Significance in The Seropositive Cases Within Selected Categorical Variables in Cats.

Variables		Infection Status			p-value
		Seropositive	Seronegative	Total	
Sex	Male	14 (15.05%)	16 (17.20%)	30 (32.26%)	0.274
	Female	37 (39.78%)	26 (27.96%)	63 (67.74%)	
Age range	Below 1 year	13 (13.98%)	15 (16.13%)	28 (30.11%)	0.418
	1—2 years	26 (27.96%)	22 (23.66%)	48 (51.61%)	
	3—4 years	9 (9.68%)	3 (3.23%)	12 (12.90%)	
	5—6 years	3 (3.23%)	2 (2.15%)	5 (5.38%)	
Habitat	Client owned	8 (8.60%)	25 (26.88%)	33 (35.48%)	0.000
	Stray	43 (46.24%)	17 (18.28%)	60 (64.52%)	

present study. Moreover, the social behavior and territoriality of cats has an impact on *T. gondii* transmission, considering that stray cats may interact with a larger number of conspecifics and have broader territories (Lepczyk *et al.*, 2015). Such interactions facilitate *T. gondii* transmission through contact and shared resources. A lack of regular veterinary care, including preventive measures and treatments for parasites, also explains the higher prevalence in stray cats compared to pet cats. Furthermore, the findings from this study emphasize the importance of managing stray cat populations and promoting responsible ownership practices with potential strategies such as trap-neuter-return (TNR) programs and public health campaigns. The results also emphasize the importance of good hygiene when handling pet cats, keeping them indoors to prevent them from hunting and eating infected prey like rodents and birds, and not feeding them raw or undercooked meat. This will enable the mitigation of risks to humans against Toxoplasmosis infection, despite the high level of interaction between humans, cats and rodents in the area.

Limitations of the Study

The Indirect ELISA technique used may have limitations because Indirect ELISA does not provide information on the genetic diversity of the *T. gondii* strains circulating in the studied populations. Understanding genetic diversity is crucial for elucidating transmission dynamics and assessing the risk of animal and human infection. Another limitation is that the prevalence of *T. gondii* infection in cats, rodents and shrews may vary temporally due to seasonal fluctuations, environmental factors, and host population dynamics. Addressing these limitations requires complementary approaches, such as molecular techniques for species-specific identification and the genotyping of *T. gondii* strains, and longitudinal studies to capture temporal variations. Despite its limitations, this study using Indirect ELISA contributes valuable data to the understanding of *T. gondii* epidemiology in cats, shrews and rodents.

CONCLUSION

In conclusion, this study has investigated the prevalence of *T. gondii* infection in rodents, shrews, and cats, shedding light on the complex interactions between these animals and the parasite. The findings from this study highlight the importance of understanding the mechanisms underlying the transmission of *T. gondii* within and between host populations. This contributes to our knowledge of the epidemiology of *T. gondii* and will help to develop effective strategies for the control and prevention of toxoplasmosis in both human and animal populations.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this study.

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ETHICAL PPROVAL

The Sokoine University of Agriculture Research Ethics Committee granted ethical clearance for this study, with reference number DPRTC/R/186/24. The Zanzibar Livestock Research Institute (ZALIRI) revised and approved the research protocols, and on February 24, 2023, the Office of the Chief Government Statistician (OCGS) and the Research Committee of the Office of the Second Vice President granted permission to conduct research in Zanzibar. The reference number for this permission is 63F74AA424AF2. Furthermore, the cat owners were verbally informed that their pets could be tested for Toxoplasmosis.

AUTHOR'S CONTRIBUTION

CMP was the principal investigator involved in the data collection, analysis, interpretation, and article writing. AAA was the co-author involved in the data collection and article writing. EMM, MJM and ASK were responsible for drafting and reviewing the article.

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