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Seroprevalence and molecular detection of Toxoplasma gondii infecting Rodents and Pigs in Iringa Municipality, Tanzania

Herman Oscar Ntungwa[1*], Elisa Mwega[2], Ernatus Martin Mkupasi[1], Abdul Selemani Katakweba[3]

Abstract

Background: Toxoplasma gondii, is an intracellular protozoan parasite that relies on both definitive and intermediate hosts, such as rodents and pigs, for completion of its life cycle. Despite its public health importance, data on its infection in these hosts is limited in Tanzania, notably the Iringa region that had reported human mortalities due to toxoplasmosis. This study was undertaken to determine the seroprevalence and molecular detection of T. gondii in rodents and pigs in Iringa municipal.

Methods: The study employed a cross-section design where 127 rodents and 240 pigs were sampled from selected wards in Iringa municipal from January 2023 to March 2023. For serological analysis, the antibody ELISA method was employed. Seropositive rodents and pigs were subjected to Nested PCR for T. gondii DNA detection using brain and blood samples from rodents and pigs, respectively.

Results: Overall seroprevalence was 1.57% for rodents and 26.25% for pigs. Among the studied wards the highest proportion of Seropositive samples was from Kitwiru (6.25%) and Nduli (40%) for rodents and pigs, respectively. Statistical analysis indicated that Pigs aged 13-24 months were significantly more likely to test positive for anti-Toxoplasma IgG antibodies (p=0.0006), and antibody detection was strongly linked to hygiene practices in pig management (p < 0.0001). Additionally, in rodents, there was a statistically significant difference in exposure status related to species and collection site, with p-values of 0.043 and < 0.0001, respectively. T. gondii DNA was detected in 100% and 3.17% of seropositive rodents and pigs, respectively. Sequencing yielded one of each for rodents and pigs PCR positive samples respectively which showed about 98.36% to 99.15% similarity with T. gondii DNA from other countries isolated from different animal species.

Conclusion: The detection of antibodies to T. gondii and subsequent identification of T. gondii DNA in samples from rodents and pigs indicate the public health significance of these animal species in the transmission of toxoplasmosis within the study region. Consequently, it is crucial to implement prevention and control measures in the studied animals to mitigate potential human exposure.

Keywords: Toxoplasma gondii, Rodents, Pigs, Seroprevalence, Molecular Detection, Nested PCR, Tanzania

Correspondence: Herman Oscar Ntungwa (hermanosca01@gmail.com)
[1]Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P. O. Box 3021, Morogoro, Tanzania
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Background

Toxoplasma gondii is an intracellular obligate parasite that is considered the most successful protozoa due to its ability to infect all warm-blooded organisms throughout the globe including humans, animals, and avian species [1]. The parasite is responsible for causing the disease known as Toxoplasmosis. The bradyzoites, tachyzoites, and sporulated oocysts are the three forms of infective stages of T. gondii. Bradyzoites are seen as tissue cysts in intermediate hosts, tachyzoites during the early infection and the definitive hosts shed non-sporulated oocysts containing sporozoites [2,3]. The sporulated oocysts are well adapted to moist environments and can survive for about two years in moist soil [4]. Sexual reproduction of the parasite occurs in the definitive hosts which essentially are members of the Felidae family mainly domesticated cats and all other warm-blooded animals such as mammals and birds serve as intermediate hosts where the asexual reproduction takes place [5]. Rodents and pigs as intermediate hosts play an important role in the transmission pathways of T. gondii to humans [6]. Toxoplasma gondii can be transmitted by rodents either through the indirect cycle or direct transmission cycle. The indirect route can be linked to the spread of contamination of released oocysts from infected cats in the environment, increasing the risk to each of the parasite hosts in the given settings, but most importantly

humans. On the other hand, rodent consumption as a source of protein by people, as is done by some communities, may result in the direct transmission of toxoplasmosis to humans. In addition, rodents may facilitate the spread of the disease to people if they are accidentally ingested by livestock. For instance, certain countries use rodents like capybaras (Hydrochoerus hydrochaeris), one of the biggest rodents in the world, and their undercooked meat might be a source of T. gondii [7-9]. Because of the intimate interactions that coexist in the environment and their significance to human health, the function is even more crucial for rodent species that are close to human habitat [10,11].

Raw or undercooked pork and products derived from it have been considered the primary foods linked to toxoplasmosis outbreaks [12]. Most of the infections in humans are meat-borne and based on limited population-based data the Food and Agriculture Organization estimated that pork being the most consumed meat contributes to about 22% of all human T. gondii infections [13].

Diagnosis of the parasite is commonly done by the Enzyme-Linked immunosorbent assay (ELISA) technique to detect antibodies specific to T. gondii in both humans and animals due to its reliability, cost considerations, and practicability [14,15]. Despite its importance, has some limitations. The most commonly reported is cross-reactivity with other protozoan parasites such as; Sarcocystis spp, Hammondia spp, Besnoitia besnoiti, and Neospora spp [16-18]. Therefore, for effective diagnosis, the ELISA method should be used together with molecular techniques like Polymerase Chain Reaction (PCR) [18]. The commonly targeted gene for PCR amplification is the B1 gene. The B1 gene is more sensitive and specific [19,20] and is considered highly conserved and a multicopy gene for T. gondii DNA detection by using various samples [19,21]. In humans, Toxoplasmosis is mostly acquired by ingestion of food containing T. gondii cysts which can be found in sheep, goats, cattle, pigs, and chickens. Also contact with body fluids such as saliva, urine, sputum, semen, and milk have been linked with the transmission of Toxoplasmosis because tachyzoites of T. gondii have been detected in body fluids of intermediate hosts [22]. The seroprevalence of the parasite reported in various studies varies between 1% and 100% globally. The differences might be due to social economic, and environmental factors, including host susceptibility, geographic location, eating habits, and health-related practices [23]. The infection rate was reported high among immunocompromised individuals such as those treated with immunosuppressive agents for cancer treatment, who have undergone organ transplants, and Human Immunodeficiency Virus-infected individuals [24-26]. The seroprevalence of more than 90% has been reported in some parts of South America and Europe [20], thus considered a major threat among food-borne infections [27]. On the other hand, less than 1% seroprevalence has been reported in some countries in the Far East [20]. Nonetheless, toxoplasmosis in humans can cause nonspecific symptoms such as fever, tiredness, headache, myalgia, swollen lymph nodes, and incoordination. In pregnant women, it can potentially lead to birth abnormalities such as abortion or stillbirth. Individuals with weakened immune systems may have more severe symptoms. Given the rising prevalence of HIV in many societies, toxoplasmosis management and prevention should emerge as a major public health priority [28]. In Africa, numerous investigations conducted among pregnant women receiving antenatal care at different healthcare units have revealed varying prevalence rates of toxoplasmosis. The prevalence ranges from 5.87% [29] to as high as 88.60% [30] in Zambia and Ethiopia respectively, across various studies resulting in an overall pooled prevalence of 51%. These findings highlight substantial public health concerns related to the disease in the region [31]. In Tanzania, studies have reported the seroprevalence of Toxoplasmosis for instance in northern Tanzania a study involving pregnant women attending the antenatal clinic at Kilimanjaro Christian Medical Centre with a prevalence of 44.5%[32], 30.9% in Mwanza [33], and 46% in Tanga [34]. A total of 188(0.08%) deaths due to toxoplasmosis have been documented in the country from 2006 to 2015 whereas the southern regions had a high case mortality rate per 100,000 populations and the Iringa region had a rate of 1.2 [35]. In Mbeya district, the study involving domestic and peridomestic rodents reported a seroprevalence of 8.7% alerting the possible public health risk in the area [36]. Despite the reported evidence of the T. gondii burden in the country in humans and livestock especially goats, sheep, and cattle, it has remained an unprioritized public health disease. Therefore, this study aimed to determine the seroprevalence and molecular detection of T. gondii in rodents and pigs, which are considered significant sources of infection to humans, to quantify its public health implications in the Iringa municipality.

Methods

Study area

The study was conducted in Iringa municipal in the Iringa region where deaths due to toxoplasmosis were recorded higher [35]. The Iringa municipal area features a diverse geography, extending northward along slopes and valleys and southward along a hilltop overlooking the Ruaha River. It is situated on a plateau at an elevation of 1,500 to 2,500 meters above sea level. Has a population of 202,490, consisting of 96,392 males and 106,098 females. The municipality lies between approximately 7.7° to 7.875° latitude south of the Equator and 35.620° to 35.765° longitude east of the Greenwich Meridian. Environmental conditions vary seasonally, with June, July, and August experiencing near-freezing temperatures [37]. The Iringa urban economy relies on agriculture, livestock keeping, and commerce, with about 40% of the population dependent on agriculture and livestock production. [38]. Wards involved in the study were Kihesa, Nduli, Mkwawa, Kitwiru, Isakalilo, Ruaha, Makorongoni, and Kitanzini (Fig.1).

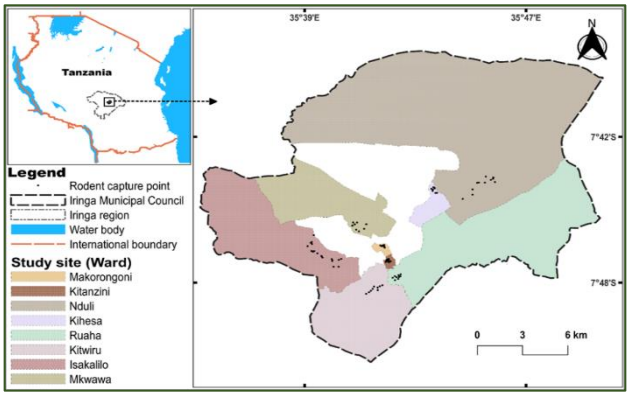

Figure 1. Map showing trapping success points for rodents captured within the visited wards in the Iringa Municipal. Source: By QGIS visited on 31/07/2023.

Study design, sampling techniques

The study involved a cross-section study design in the Iringa Municipal from January 2023 to March 2023. A total of eight randomly selected wards were involved in the study. For rodents all randomly selected wards were visited but for pig sampling the six wards were involved after excluding the two wards that had no pigs and had no records of keeping pigs as the information was obtained from the municipal council livestock officer. On the other hand, rodent traps were set in households that reported complaints of rodent infestation in their houses and the surrounding environments upon interviewing them.

Sample size determination

The sample size for establishing the seroprevalence of T. gondii in rodents and pigs was calculated according to the formula: n=Z2 p (1-p)/ d2. Where; n=sample size, expected prevalence, d= desired precision 5%, Z=statistics corresponding to the level of confidence=1.96. Therefore, for rodents, the sample size required for this study was 87 rodents considering the global seroprevalence of T. gondii in rodents which was reported to be 6% from the previous studies [9]. However, the sample size was increased to 127 considering the differences in population density, distribution, and trapping success of rodents in different areas with varying climatic conditions. On the other hand, the sample size for establishing the seroprevalence in pigs was calculated based on the reported average prevalence (p) of 19% around the globe [39]. The number of pigs expected to be sampled was 237 but to get an equal number of 40 pigs from each ward selected for the study, the sample size was increased to 240 pigs.

Capturing and identification of rodent

Rodents were captured in households, cereal storage facilities, cultivated land, and fallow fields. Locally made live wire traps baited with feeds such as fish, tomatoes, and sardines were used in households and storage facilities for three consecutive days to increase capture rates [40]. On farmlands and fallow fields, about 100 Sherman traps which were baited with a mixture of peanut butter and maize flour, set in 10 lines with 10 stations each, spaced 10m apart for two consecutive nights. Traps were set in the evening and inspected in the following morning. The GPS for each successful trapping location was recorded [41], and subsequent identification of captured rodents was followed using taxonomic keys by Happold et al. [42].

Sample collection

Samples collected from rodents were blood and brain tissues whereas in pigs only blood samples were collected. In rodents, the intracardiac puncture route was used to collect about 3mls from each captured rodent [43]. Rodents were humanely euthanized by the use of Halothane and the brain tissue samples were obtained after dissecting and opening the skull to expose the brain and then extracted by using tissue forceps into cryo-vials each containing absolute alcohol (ethanol 99.8%). The brain sample was immediately transported to the laboratory and stored at -20°C for subsequent use in molecular analysis. On the other hand, in pigs, whole blood was collected through the external jugular vein following standard operating procedures for blood collection [44]. The whole blood samples collected from rodents and pigs were left to settle and then centrifugation followed at 5000 rpm for 5 minutes at room temperature (22°C) to extract serum. The extracted sera sample was then immediately stored at -22°C for future serological use [45].

Serology technique

The indirect Enzyme-Linked immunosorbent assay technique was used with the aid of the pig-type® Toxoplasma Ab commercial kit by INDICAL BIOSCIENCES GmbH, Deutscher Platz 5b,04103 Leipzig, Germany for detecting anti-T. gondii IgG-specific antibodies for all sera samples from rodents and pigs. All manufacturer's recommendations were followed to accomplish the test procedures. An ELISA plate analyzer (MICRO READ 1000, Inqaba biotech™) was used to measure the Optic Density (OD) at 450 nm. The OD value correlates with the concentration of anti-Toxoplasma antibodies within the test sample. According to the manufacturer's protocol. Results were considered valid if the Mean Value (MV) of the measured OD value for the positive control (PC) was ≥ 0.7 and the Mean Value (MV) of the measured OD value for the Negative Control (NC) was ≤ 0.25 (using the short protocol where sample incubation was done for 60 minutes at Room temperature equivalent to 22°c). For each test sample, the results were calculated employing the formula; S/P= (ODsample-MV ODnc)/ (MV ODpc-MV ODnc). Where: S/P is the ratio of sample OD to mean OD of the positive control, ODsample is the Optic Density of the sample, MV ODnc is the Mean Value of Optic Density of the Negative Control and MV ODPC is the Mean Value of Optic Density of the Positive Control. Samples with an S/P ratio of ≥ 0.3 were considered positive meaning that specific antibodies to T. gondii were detected and samples with an S/P ratio < 0.3 were considered negative meaning that specific antibodies to T. gondii could not be detected.

Molecular technique

DNA Extraction

Following a procedure stipulated by Wang et al. [46], total DNA extraction from tissues (whole blood and brain) was carried out. ZYMO Research's Quick-DnaTM Universal Kit was utilized for extraction, where 0.025g of each tissue sample was mixed with 95 µl of water, 95 µl of solid tissue buffer, and 10 µl of proteinase K solution before being heated at 55 °C for 3 hours to improve its solubility. The sample was then centrifuged at 12000xg for one minute, and the supernatant was transferred to a clean tube. Then, each 200µl of transferred supernatants was mixed with 400 µl of genomic binding buffer. This mixture was transferred to a Zymo-spinTM IIC-XL column attached to a collecting tube, and it was then centrifuged at 12000 x g for one minute. Several wash buffers were used during the DNA purification processes, and subsequent centrifugation as directed by the protocol. An elution buffer was then used to elute the pure DNA extract. On the other hand, DNA extraction from whole blood followed the same protocol with variations at the first stage where a larger volume (20µl) of proteinase K, 200µl of biofluid, and cell buffer (unlike tissue buffer), were added to 200µl of blood sample then mixed thoroughly and incubated at 55 °C for 10 minutes unlike 1-3 hours required to incubate DNA extracted from tissues. Before storage extracted DNA was subjected to a NanoDropTM spectrophotometer for concentration and quality measurement.

For subsequent investigation, the final DNA extract was kept in a freezer at -20°C.

Molecular identification of the parasites

PCR amplification reaction

The extracted DNA was amplified via conventional PCR in a 25µl reaction volume containing 5µl of concentrated bioneer premix, 1µl of both forward and reverse B1 primers shared equally, and 14µl of nuclease-free water, and 5µl of purified DNA into the 35-fold B1 gene of T. gondii targeting 530bp. Nested PCR was employed under the following PCR conditions; for the first round and Nested round respectively; Initial denaturation of the DNA template was done at 940C for 5 minutes followed by 35 PCR cycles, then final denaturation at 940C for 1 minute, primer annealing at 520C for 1minute followed by initial elongation at 720C for 1minute and final elongation was at 720C for 10miutes and the second round (nested) same amplification condition was employed [47]. Amplification was done with the aid of the following primers, first-round forward primer 5'-TGTTCTGTCCTATCGCAACG-3', Reverse primer 5'-ACGGATGCAGTTCCTTTCTG-3' Second round primer; Forward primer 5'-TCTTCCCAGACGTGGATTTC-3'; Reverse primer 5'-CTCGACAATACGCTGCTTGA-3'. The same conditions were also set for the second round (nested) using second-round primers as listed above, PCR reaction was carried out in a 25µl reaction volume containing 5µl of concentrated bioneer premix, 1µl of both forward and reverse B1 primers, and 18µl of nuclease-free water and 1µl of the amplified amplicons (PCR product) of the first round. The amplicons were separated by 1.5% agarose gel-stained ethidium bromide (gel electrophoresis) and then visualization and documentation were done by gel doc (Vilber Lourmat machine) [48].

Sequencing and evolutionary phylogenetic tree

Nested PCR–positive samples for T. gondii DNA (B1-gene) were sent to Macrogen Inc., Seoul, Korea, for sequencing. By using the NCBI BLAST software, the obtained sequences were further analyzed to determine the degree of similarity with other T. gondii isolates from other animal species in other regions of the world. Molecular Evolution Genetics Analysis (MEGA) version 7.0 was used to align the dataset for the B1 gene of T. gondii. Obtained nucleotide sequences in the current study were deposited in the GenBank database and given accession numbers OR051759 and OR051760. The neighbor-joining method was used to construct the phylogenetic tree of aligned sequences aiming at establishing the evolutionary relationship of the obtained nucleotides and others isolated from other parts of the globe [49].

Data analysis

Collected data were entered into Microsoft Excel 2017 and then SAS analytic software was employed for analysis. Seroprevalence for individual tested samples was estimated from the ratio of ELISA-positive sera to the total number of analyzed samples, with 95% exact binomial confidence intervals (95% CI). Associations between serological results and independent variables such as sex, species, habitat, and wards for rodents, and pigs; breed, sex, hygiene status, and wards were determined by Pearson's chi-square or Fisher's exact tests, as appropriate. At p-value < 0.05 the differences were considered statistically significant.

Results

Rodents and pig's species /breeds composition

A total of 127 rodents involving three species were captured and sampled during the study. However, the total capture was dominated by Rattus rattus species from households and the least was Mus musculus captured in tomato farms in the vicinity of human dwellings. Besides rodents, a total of 240 domesticated pigs (Sus domesticus) were sampled. The pigs comprised crossbreeds of local and largely white, Local and Landrace, Local and Saddleback, and pure Duroc pigs (Table 1).

Seroprevalence of T. gondii in Rodents and Pigs

Of the 127 serum samples tested from rodents, two tested positive for T. gondii, with an overall seroprevalence of 1.57% whereas 63 (26.25%) of the 240 pigs tested positive for anti-T. gondii IgG-specific antibodies. In all wards, seropositive rodents were detected in only one ward while seropositive pigs originated from all sampled wards and ranged from 15% to 40% (Table 2).

Risk factors for T. gondii seropositivities in rodents and pigs

In rodents, differences in seroprevalence among sexes and wards were not significant with p-values of 0.1142 and 0.536, respectively. The differences in seroprevalence among pig breeds, sexes, and wards of origin were insignificant with p-values of 0.5252, 0.2694, and 0.1101, respectively. On the other hand, the differences in seroprevalences were found to be statistically significant among various age groups (p-value <0.0001). Additionally, the detection of anti-T. gondii IgG-specific antibodies were found to be statistically significant in relation to hygiene status in pig management with a p-value of < 0.0001(Table 3).

Molecular detection of T. gondii infection in rodents and pigs

Polymerase chain reaction (PCR) analyses

Figure 2 shows that two brain samples from ELISA seropositive rodents were presented for PCR analysis, in both (2/2, 100%) samples, T. gondii DNA was detected targeting the B1 gene at 530bp. Furthermore, the T. gondii DNA was detected in two samples out of the 63 seropositive tested pig samples (2/63, 3.17%).

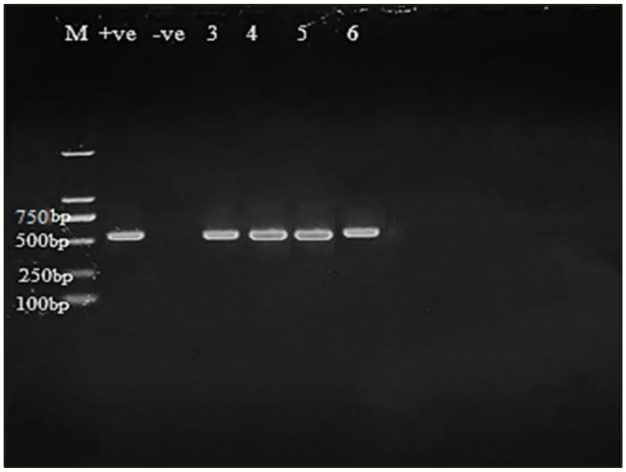

Figure 2. PCR amplification of T. gondii B1 gene; Where M is a marker 250kb and lanes 3-6 are samples, where lanes 3-6, are positive samples located at 530bp and lanes 1 and 2 are positive and negative controls respectively.

Sequence and evolutionary phylogenetic analysis

From the four T. gondii DNA-positive samples, two good amplicons upon sequencing were obtained. One was among the positive PCR samples of rodents (OR051760) and the other was from positive PCR samples of pigs (OR051759). The degree of similarity was 98.36% - 99.15% when the obtained sequence nucleotides in this study were compared with other sequences in the NCBI database isolated from other parts of the world involving animals like black bears (Ursus americanus), mussels, humans, sheep, camel, birds, and cows. The sequences discovered in this investigation had 98.52%, 98.74%, and 98.95% similarity with those previously reported in Australia, North India, and Western Australia isolated from domestic cats, goats, and little penguin birds respectively. According to evolutionary relationship analysis, these two sequences grouped along with other T. gondii ranging from less-pathogenic and highly pathogenic strains, showing no type-specific differentiation. Consider Fig. 3 below.

Table 1: General Overview of Sampled Rodents and Pigs (site of capture)

Species /Breed	Household	Storage facility	Maize farm	Tomato farm	Total N (%)
Rattus rattus	81	10	4	0	95(74.8)
Mastomys natalensis	1	0	20	10	31(24.41)
Mus musculus	0	0	0	1	1(0.79)
	82	**10**	**24**	**11**	127(100)
Local and Large white					128(53.3)
Local and landrace					83(34.6)
Local and saddleback					26(10.8)
Duroc					3(1.3)
Total					**240(100)**

Table 2: Ab-ELISA results for T. gondii infection in rodents and pigs

Type	Breed/Species	Total tested	Tested positive	Tested negative	Seroprevalence N (%)
Rodents	Rattus rattus	95	0	95	0(0)
	Mastomys natalensis	31	2	29	2(1.57)
	Mus musculus	1	0	1	0(0)
Total		**127**	**2**	**125**	**2(1.57)**
Domestic pigs	Local and Large white	128	37	91	37(28.9)
	Local and landrace	83	21	62	21(25.3)
	Local and saddleback	26	5	21	5(19.2)
	Duroc	3	0	3	0(0.0)
Total		**240**	**63**	**177**	**63(26.25)**

Discussion

In Tanzania, this is the first study to elucidate the seroprevalence and molecular identification of T. gondii infecting pigs raising concerns about potential public health risks, particularly for consumers who prefer undercooked pork. The study also reports on the seroprevalence of the infection in rodents. The findings of the study in rodents are in line with the study in Niger that reported 1.96% seroprevalence [50] and South Korea 1.49% [51], but half of the seroprevalence values reported in Senegal 3.56% [52] and Switzerland 4.23% [53] and two times that reported in China [9], Grenada 0.84% [54] and Scotland 0.94% [9]. Apart from that, the highest seroprevalence of the infection has been recorded in countries like Israel 77.78% [55], and in Brazil 61.54% [56]. The differences in seroprevalence values in these countries could be explained by differences in climatic conditions, abundances of definitive hosts, environmental factors, sensitivity of the serological method used, and susceptibility to infection between species or vertical transmission variability [57]. For example, congenital transmission has been recorded to be high among rodent species of wild rat populations regardless of environmental contamination. This may explain the lower seroprevalence in our current study considering that a large number of rodents involved were captured from households and few from peridomestic areas in close vicinity to human settlements which may be considered to have a low level of congenital transmission and thus low prevalence levels in such species [58]. Statistical analysis revealed the absence of a significant relationship between tested rodents and toxoplasma exposure in terms of sex and wards. This implies that both male and female rodents from all wards of the Iringa municipal had an equal chance of being infected with T. gondii. The same observation was reported in Pakistani [59] and Bangladeshi [60]. The serological tests especially those targeting antibodies may underestimate or overestimate the prevalence of the infection. This suggests that seroprevalence estimates may not accurately reflect the situation of infection status among rodents in an area [61, 9]. The reason could be that the generation of antibodies can be influenced by many factors including host age, host immunity, parasite genotype, and infection persistence. In certain species, the quantity of antibodies generated decreases quickly and loses their binding sites becoming unrecognizable. Additionally, some rodents that have been congenitally infected do not generate antibodies despite some of their tissues being accumulated with T. gondii parasites [62,63]. Therefore, the absence of anti-T. gondii IgG-specific antibodies in the two species (R. rattus and M. Musculus) in the current study do not

mean the absence of exposure to the infection in the study area. The same observation was reported in Thailand in a survey of toxoplasmosis in rodents [64].

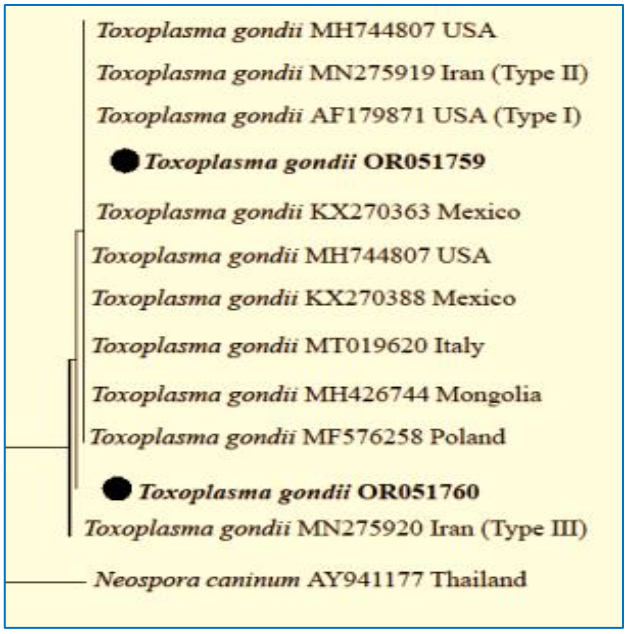

Figure 3. Phylogenetic tree construct of the T. gondii (B1-gene) from rodents and pig samples collected in Iringa municipal, Tanzania. Cycles marked with black boldface represent sequences obtained in this study and the accession number of T. gondii samples from the Gen Bank on each line is labeled. The neighbor-joining technique was used for analysis with 1,000 bootstrapped replicates.

Because has been revealed that T. gondii infection in rodents can alter their behavior array such as losing their innate fear of cats, increased attraction to cats' urine, and impairment of neurons. These increase the possibility of cats eating infected rodents [65,57]. However, molecular detection of T. gondii in rodents in this study is consistent with previous studies that have reported molecular confirmation of the parasite in similar hosts in countries like Brazil [56], Grenada [63], Bangladesh [60], and Pakistani [59]. Contrary to this study, studies in Sweden [61]and Poland [66] did not detect T. gondii DNA using the PCR technique. This can be attributed to the differences in the sensitivity and specificity of detection methods employed in these different studies. Phylogenetic analysis revealed that the obtained sequence from rodents formed a cluster with other T.gondii from animal species ranging from terrestrial and aquatic environments confirming the presence of T.gondii in sampled animal species and therefore cementing the evidence of the cosmopolitan nature of infection of the parasite [67, 68]. Nevertheless, the findings in pigs in this study indicate the public health potential of the host in the triad of T. gondii and are in line with other serological studies conducted elsewhere such as in Iberian pigs in Spain at 21.9% [69], in Cuba 22.2% [70] and 25.4% in Poland [71]. Contrary to the findings reported in Romania at 46.8% [72] and in the Podkarpackie region in Poland at 32.6% [73]. The differences could be due to the variation of environmental factors supporting the proliferation of the parasite. The lower seroprevalence of T. gondii was significantly associated with lower mean annual temperature and higher geographical latitude [39]. The age of the pigs was found to influence the infection seropositivity. The study observed an increase of seropositivity with an increase in pig age and higher seroprevalence values were observed among pigs of age group 13-24 months. Anti-T. gondii IgG-specific antibodies can persist for lifelong in an animal's body therefore older pigs may have long periods of exposure to T. gondii oocysts excreted by cats in the environment and interaction with other intermediate hosts such as rodents which may be harboring tissue cysts. Also decrease in immunological function, termed immunosenescence, is frequently linked to aging. Therefore, the capacity to develop a successful immunological response in older pigs against T. gondii becomes compromised, raising the risk of seropositivities and persistent infection [74,75]. Similar results were reported by previous investigations in Switzerland [76] and Poland [73].

Hygiene status in pig management in the current study has been significantly associated with T. gondii exposure to pigs, where pigs reared under poor hygiene showed higher seroprevalence values than that observed in pigs managed under good hygiene practices. Poor hygiene on pig farms has been linked to sporulated oocyst contamination of the soil, feed, and water, which can lead to the transmission of T. gondii and increase the percentage of pigs that test positive for specific antibodies to T. gondii in the given pig farm. The T. gondii can be spread to pigs by poor handling and storage techniques, such as keeping feed in places where it can be contaminated by infected cat feces or utilizing contaminated water sources [77-79].

Additionally, it has been noted that poor hygiene practices including incorrect waste management and disposal or insufficient rodent control measures might hasten rodent infestations, which are the intermediary hosts for T. gondii and whose existence in pig farms can aid in the parasite's spread [80]. However good management practices and biosecurity measures including good hygiene in feed processing and storage facilities, effective rodent control programs, denying access to cat and cat feces, and provision of uncontaminated water to livestock such as pigs can potentially reduce T. gondii exposure and subsequent infection to animals whereas the infection rates may be high in poorly managed pig farms. The same observation was reported in southern Piauí [81] and in Cuba [79]. This study further confirms that for precise diagnosis of toxoplasmosis, the ELISA diagnostic techniques should be used in combination with molecular techniques like PCR. The molecular technique in this study was employed on sixty-three blood samples of seropositive pigs targeting the B1 gene. The results of the study are similar to those reported in a study involving domestic pigs in Cuba that reported a 4% T. gondii DNA detection rate in seropositive sampled pigs [72]. Contrary to the DNA detection rate in pigs other countries such as Ireland [82], Italy [83], North India [84], Poland [73], and southwest China [85] reported higher prevalence. Analysis of the phylogenetic tree of the B1 gene revealed the sequences that formed one cluster with sequences detected in different parts of the world in various animals both terrestrial and aquatic species. Terrestrial animals included cows in Iran [86], Bactrian camels in Mongolia[87], sheep in Mexico[88], black bears (Ursus americanus) in the USA[89], and humans in Iran [90]. Aquatic species formed one group with the bivalve mollusks known as mussels [86]. The findings of the study provide further evidence for the existence of infection in animal species under the study and less host specificity of T. gondii plus its global distribution nature.

Conclusion

The current study highlights the prevalence of T. gondii parasites among the animals studied. This was achieved by detecting anti-T. gondii IgG-specific antibodies, revealing T. gondii exposure in a significant proportion of rodents (1.57%) and pigs (26.25%). Additionally, T. gondii DNA was confirmed in both animal species. These findings provide compelling evidence that both rodents and pigs in Iringa municipal may play a role in the transmission cycle of T. gondii to humans. Furthermore, the identification of T. gondii DNA in pig samples raises concerns about potential public health risks, particularly for consumers who prefer undercooked pork. Consequently, it is imperative to conduct further investigations focusing on other intermediate hosts, such as goats, sheep, cattle, and chickens, which are commonly used as protein sources in the area.

Table 3. Relationship between T. gondii seropositivity status and selected variables

Variable		Tested negative	Tested positive	Total N (%)	Chi-square	P-value
Species	Rattus rattus	95	0	95(74.8)	6.2926	0.043
	Mastomys natalensis	29	2	31(24.4)		
	Mus musculus	1	0	1(0.0079)		
Sex	Female	70	0	70(55.12)	2.4954	0.1142
	Male	55	2	57(44.88)		
Site	Household	82	0	82(64.57)	21.4284	< 0.0001
	Maize farm	24	0	24(18.9)		
	Storage facility	10	0	10(7.87)		
	Tomato farm	9	2	11(8.7)		
Wards	Isakalilo	19	0	19(14.96)	6.0325	0.536
	Kihesa	10	0	10(7.87)		
	Kitwiru	30	2	32(25.2)		
	Kitanzini	8	0	8(6.3)		
	Makorongoni	14	0	14(11.02)		
	Mkwawa	20	0	20(15.75)		
	Nduli	14	0	14(11.02)		
	Ruaha	10	0	10(7.87)		
Breed	Local and Large white	91	37	128 (53.33)	2.2346	0.5252
	Local and landrace	62	21	83 (34.58)		
	Local and saddleback	21	5	26 (10.83)		
	Duroc	3	0	3 (1.25)		
Sex	Female	104	42	146 (60.83)	1.22	0.2694
	Male	73	21	94 (39.17)		
Age	0-12 Months	75	10	85 (35.42)	14.6977	0.0006
	13-24 Months	89	48	137 (57.08)		
	25-36 Months	13	5	18 (0.075)		
Hygiene status	Good hygiene	147	22	169 (70.42)	51.6673	<0.0001
	Poor hygiene	30	41	71 (29.58)		
Wards	Isakalilo	34	6	40 (16.67)	8.975	0.1101
	Kihesa	27	13	40 (16.67)		
	Kitwiru	33	7	40 (16.67)		
	Mkwawa	29	11	40 (16.67)		
	Nduli	24	16	40 (16.67)		
	Ruaha	30	10	40 (16.67)		

Abbreviation

Ab: Antibody; AIDS: Acquired Immunodeficiency Syndrome; Bp: Base pairs; CI: Confidence interval; DF: Degree of freedom; DNA: Deoxyribose-Nucleic Acid; ELFA: Enzyme-Linked Fluorescent Assay; ELISA: Enzyme-Linked Immunosorbent Assay; FAO: Food Agriculture Organization; GIS: Geographic information system; GPS: Geographical Position System; HIV: Human Immunodeficiency Virus; IFAT: Immune Fluorescent Antibody; IgG: Immunoglobulin gamma; KCMC: Kilimanjaro Christian Medical Centre; MEGA: Molecular Evolution Genetics Analysis; MEIA: Microparticle enzyme immunoassay; MV: Mean value; NBS: National Bureau of Statistics; NC: Negative Control; NCBI: National Center for Biotechnology Information; OD: Optic Density; PC: Positive Control; PCR: Polymerase Chain Reaction; Rpm: Revolution Per Minute; SAS: Statistical Analysis System; Spp: Species; SUA: Sokoine University of Agriculture; TMA: Tanzania Metrological Authority; TVLA: Tanzania Veterinary Laboratory agency; URT: United Republic of Tanzania; WEO: ward Executive Officer; WHO: World Health Organization; ZVC: Zonal Veterinary Centre

Declaration

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Availability of data and materials

Data will be available by emailing herman.ntungwa@sua.ac.tz

Authors' contributions

Herman Oscar Ntungwa is a principal investigator involved in data collection, analysis, interpretation, and article writing. Elisa Mwega (EM), Ernatus M. Mkupasi (EMM), and Abdul S. Katakweba (ASK) are supervisors, all supervisors were responsible for drafting and reviewing the article.

Ethics approval and consent to participate

This research was conducted following the Declaration of Helsinki. The Institutional Review Board of the Sokoine University of Agriculture provided an ethical clearance with reference number SUA/DPRTC/R/186/67. Additionally, approval to conduct the study in the selected wards in the district was granted by the Iringa Municipal District Council administrative authorities, with reference number IMC/T.40/39/82 issued on 13 February 2023.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

Author Details

[1]Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P. O. Box 3021, Morogoro, Tanzania.

[2]Department of Veterinary Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, P.O. Box 3019, Morogoro, Tanzania.

[3]Institute of Pest Management, Sokoine University of Agriculture, P. O. Box 3110, Morogoro, Tanzania.

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