

Use of the nested polymerase chain reaction for detection of *Toxoplasma gondii* in slaughterhouse workers in Thika District, Kenya

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Background. The widely used methods of diagnosis of *Toxoplasma gondii* are serological. Current reports indicate a high seroprevalence of *T. gondii* in humans in Kenya. There is a need for more sensitive diagnostic tests, especially when the specific antibody titres are below detectable threshold levels. Use of the polymerase chain reaction (PCR) targeting the repetitive 529 base pair loci has been reported to be sensitive and specific.

Objective. To detect *T. gondii* in a high-risk group of public health workers in Thika District, Kenya.

Methods. In total, 87 human blood samples were collected from male slaughterhouse workers between 1 March 2013 and 25 June 2013. The DNA extracted was amplified by the nested PCR.

Results. *T. gondii* was detected in 39.1% (34/87) of the workers. In the cow-sheep-goat slaughterhouses the prevalence ranged between 20% and 60%, while all the chicken slaughterhouse workers (6/6, 100%) tested positive. The difference in *T. gondii* positivity between the workers in the chicken slaughterhouse and those in the cattle-sheep-goat slaughterhouses was statistically significant ($p=0.003$).

Conclusion. This study shows the presence of *T. gondii* in an asymptomatic high-risk group in Thika District, indicating the need for enhancement of public health awareness.

S Afr Med J 2016;106(4):417-419. DOI:10.7196/SAMJ.2016.v106i4.8777

Toxoplasma gondii is an intracellular protozoan parasite that infects a wide range of warm-blooded animals. The parasite causes toxoplasmosis in humans, which manifests as mild nonspecific infection or may be asymptomatic in immunocompetent hosts.^[1] In immunocompromised humans *T. gondii* infection may lead to cerebral toxoplasmosis.^[2] In addition, congenitally acquired toxoplasmosis can have severe and life-threatening consequences such as abortion, stillbirth, abnormal enlargement or reduction in child head size, mental retardation, vision loss and seizures.^[1,3]

The keeping of livestock (pigs, cows, poultry and goats) in highly populated urban areas, as occurs in Thika District, increases the risk for transmission of zoonoses such as toxoplasmosis.^[4,5] A recent study of farmers in Thika District identified potential risk factors for transmission of toxoplasmosis, including the keeping of cats allowed to range freely outside the home (the cat being the definitive host of *T. gondii*), improper handling and disposal of cat faeces (by the few owners who provided cat litter boxes) and livestock manure, and consumption of untreated water.^[6]

The current diagnostic methods are mainly serological. Studies of the prevalence of toxoplasmosis in Kenya are few. One report indicates prevalence of between 35% and 60% in preschool children.^[7] The use of nested polymerase chain reaction (nPCR) has been shown to be more sensitive and specific for detection of *T. gondii* than the serological tests.^[8] The targeted genes include 18S ribosomal DNA, the B1 multicopy element, *SAG 1*, *SAG 2* and the 529 base pair (bp) multicopy element. Previous studies have shown that the 200 - 300-fold 529 bp repeat element is a reliable target.^[9] Using this target, we determined the presence of *T. gondii* in slaughterhouse workers in Thika District.

Methods

Study group

The study group comprised licensed abattoir workers in Thika District, which covers 1 960.2 km² and lies between 3°53' and 1°45' S and 36°35' and 37°25' E.

Sample collection and processing

Using a standard venepuncture technique, 4 mL human blood samples were collected from consenting workers. The blood was transferred to labelled sterile tubes, which were immediately stored in an upright position in a refrigeration coolbox. The blood samples were transported to the laboratory within 5 hours of collection, and stored upright at +4°C overnight before separation of serum. Serum was separated from the blood samples by pipetting and samples were stored in aliquots of 200 µL at -20°C until analysed.

Ethical approval

Permission to undertake the study was obtained from the institutional ethical review committee of Kenya Medical Research Institute. The workers were informed about the study, and those who gave written consent were included.

DNA isolation

DNA was extracted from the serum samples using Quick-gDNA™ MiniPrep kit (Zymo Research, USA) according to the manufacturer's instructions. The extracted DNA was stored at -20°C in DNase-free Eppendorf tubes.

Positive control

Reference *T. gondii* (RH) DNA was kindly donated by the Friedrich-Loeffler-Institut Bundesforschungsinstitut für Tiergesundheit in Germany, through the assistance of Dr Gereon Schares.

PCR technique

Nested PCR targeting the 529 bp multi-copy gene element (GenBank Accession No. AF146527) was performed as previously described.^[10] The reaction mixture for primary amplification was optimised in 10 µL reaction volume. The components of the PCR reaction mix included 2 µL of 10 × Dream Taq buffer (Thermo Scientific, USA), 0.15 µL of 5 µM of each of the primer pair 5'TGACTCGGGCCAGCTGCGT3' and 5'CTCCTCCCTTCGTCCAAGCCTCC3', 0.5 µL of Dream Taq polymerase (Thermo Scientific, USA), 0.5 µL of DNA template, and 0.2 µL of 10 mM dNTP mix; the reaction volume was then topped with double-distilled water. The PCR reaction was run for 30 cycles of denaturation temperature at 94°C for 1 minute, annealing at 58°C followed by an extension at 72°C.

In the secondary amplification, the primary PCR amplification product was diluted 1:99 using double-distilled water. From the dilution product, 0.5 µL was used as template. The reaction mixture was composed of 2.1 µL of 10 × Dream Taq buffer (Thermo Scientific, USA) and 0.5 µL of 5 µM of each primer pair 5'AGGGACAGAAGTCGAAGGGG3' and 5'GCAGCCAAGCCGGAACATC3'. A volume of 1 µL of Dream Taq polymerase (Thermo Scientific, USA) was added and the reaction volume topped up to 10 µL with double-distilled water.

PCR was then run for 35 cycles of denaturation temperature at 94°C for 1 minute, annealing at 52°C, followed by an extension at 72°C. The reference *T. gondii* (RH) DNA was used as positive control, while PCR water was used as the negative control. The two controls were included in every amplification step.

The product generated in the second amplification was run in 1.5% agarose gel prestained with 3 µL of ethidium bromide (1 µg/mL).

Statistical analysis

Fisher's exact test was used to determine significance of differences in *T. gondii* positivity between the chicken slaughterhouse workers and the cattle-sheep-goat slaughterhouse workers. Statistical analysis was performed with Epi-Info software (version 6, Centers for Disease Control,

USA); *p*-values <0.05 were considered to be significant.

Results

The primary PCR product was characterised by multiple bands of various sizes, sharp and faint bands equivalent to 400 bp being observed in some samples and the positive control, as shown in Fig. 1. The secondary amplification product clearly showed the predicted amplicon size of 164 bp as shown in Fig. 2.

In six of the eight slaughterhouses, workers tested positive for toxoplasma DNA (Table 1). None of the workers in the Mundoro and Gachika slaughter slabs tested positive. The chicken slaughterhouse recorded the highest prevalence, followed by the Thika slaughter slab with 60.0%. Overall, 39.1% (34/87) of the workers tested positive for *T. gondii*.

The prevalence of *T. gondii* among the workers (*n*=6) in the chicken slaughterhouse was significantly higher than among workers (*n*=81) in the seven cattle-sheep-goat slaughterhouses (100% v. 34.6%; *p*=0.003), when these were compared as two different study sites based on type of livestock slaughtered.



Fig. 1. Gel photograph of primary PCR product showing bands of various sizes. The human serum samples are shown by lanes 1 - 16. NC, PC and M denote the negative control, positive control and 100 bp ladders, respectively.

Discussion

This is the first study on the prevalence of *T. gondii* in a suburban setting (Thika District, Kenya). The study demonstrated that up to 39.1% of the slaughterhouse workers were infected with *T. gondii*, as detected using nPCR. Higher seroprevalences of *T. gondii* have been reported in school-children (60%) and human blood donors (54%) in Nairobi, Kenya.^[7,11] Earlier studies in Tanzania have reported *T. gondii* infection among abattoir workers and livestock keepers,^[12] and may indicate an occupational risk of *T. gondii* transmission. In Poland, high *T. gondii* seroprevalences have been recorded in abattoir workers from meat factories in Lublin.^[13] Among the three slaughter divisions, the Cured Meat Division recorded the highest rate (76.2%), followed by the Meat Production Division (66.6%) and the Slaughter Division (46.1%), compared with a reference blood donor group with a seroprevalence of 55.7%.^[13] In the current study, the highest prevalences of *T. gondii* infection were noted in the Ruiru and Thika abattoirs. These are in urban areas with mixed economic activities including keeping of livestock such as cattle, sheep, goats and chickens. The two sites are also characterised by clustered dwellings because of their high population density, compared with Mundoro, Gachika and Gathage, which

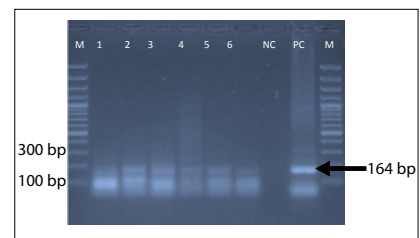


Fig. 2. Gel photograph for secondary PCR amplification product. The human serum samples are represented by lanes 1 - 6. NC, PC and M denote the negative control, positive control and 100 bp ladders, respectively.

Table 1. Male workers who tested positive for *T. gondii* DNA using nPCR

Study site	Type of livestock slaughtered	Workers testing positive n/N (%)
Chicken slaughterhouse	Chicken	6/6 (100.0)
Thika slaughter slab	Cattle, sheep, goats	6/10 (60.0)
Ruiru slaughterhouse	Cattle, sheep, goats	10/22 (45.5)
Juja slaughter slab	Cattle, sheep, goats	6/15 (40.0)
Municipal slaughterhouse	Cattle, sheep, goats	5/14 (35.7)
Gathage slaughter slab	Cattle, sheep, goats	1/5 (20.0)
Mundoro slaughter slab	Cattle, sheep, goats	0/10 (0.0)
Gachika slaughter slab	Cattle, sheep, goats	0/5 (0.0)

are rural sites. Risk factors for *T. gondii* transmission in the urban setting may therefore be more concentrated than in the rural areas as a result of environmental contamination.

The significantly higher infection level in chicken slaughterhouse workers may indicate higher exposure to *T. gondii*. Free-range chickens feed off the ground and are a good indicator of the oocysts in soil. Chickens are in fact increasingly being identified as important sources of *T. gondii* in Kenya^[14] and in other developing countries.^[15] New toxoplasma infections in slaughterhouse workers may be minimised by thorough washing of hands and working tools after handling animal carcasses, especially chickens. Meat and meat products should be sufficiently cooked or processed to destroy the infective stages of *T. gondii*.

The slaughterhouse workers were asymptomatic. *T. gondii* infection in immunocompetent humans is usually asymptomatic and therefore remains unrecorded.^[16] One report has shown that nearly 25% of Ugandan HIV patients with neurological complications tested positive for toxoplasmosis as a result of parasite reactivation.^[17] The prevalence of HIV among adults aged 15 - 49 years in Thika District is around 6%, ranking 11th of 47 Kenyan districts.^[18] As a result of the high HIV prevalence in the study area, there is a higher possibility of reactivation of the parasite, leading to severe outcomes. This study and previous ones^[7,11,19] indicate the need for strategies to control toxoplasmosis in Kenya.

In this study, *T. gondii* DNA was detected by nPCR. Serological methods have limitations such as long and laborious test procedures and low sensitivity due to low antibody levels.^[20] Detection of *T. gondii* DNA in blood samples by nPCR, based on the multilocus 529 bp element, was found to be more sensitive than targeting B1 repeat loci, and moreover was highly specific.^[10] This finding showed that blood samples can reliably be used as alternative samples for toxoplasmosis studies by PCR, as suggested previously in ocular toxoplasmosis studies.^[21] However, the sensitivity of PCR may be limited by the random distribution of the parasite and varying parasite densities in affected tissue.^[22] Currently there is no consensus on test protocols and sensitivity for clinical diagnosis of toxoplasmosis by PCR using various samples,^[23] and it has therefore been suggested that there is a need to develop more acceptable reference guides for the performance and result validation of PCR techniques.^[24] The cost of PCR is high and its application under field conditions is limited by the need for a thermocycler. Alternative amplification of DNA by loop-mediated isothermal amplification, using the 529 element, has been determined^[10,25] and should be developed further for application in point-of-care diagnosis.

Conclusion

In Thika District, up to 39.1% of slaughterhouse workers are infected with *T. gondii*, with the highest infection rate being recorded in the chicken slaughterhouse workers. This study implies a need for public health intervention to limit environmental contamination.

Acknowledgement. This research was supported by the African Union and the government of the Republic of Kenya. We acknowledge Gereon Schares at the Friedrich-Loeffler-Institut Bundesforschungsinstitut für Tiergesundheit in Germany for providing the toxoplasma RH-positive control. We also thank Dawn Maranga and Samson Mutura at the Institute of Primate Research for technical assistance.

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Accepted 18 March 2015.