



Communication

# Seroprevalence of *Toxoplasma gondii* in Slaughtered Pigs in Kiambu, Kenya

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**Simple Summary:** There is a rapid increase in the production and consumption of pork, particularly in low- and middle-income countries. In Kenya, the demand for pork meat is driven by expanding and urbanizing human populations. The objective of this study was the detection of *Toxoplasma gondii* antibodies in pigs slaughtered for human consumption within peri-urban areas of Nairobi and Kiambu Counties in Kenya. *Toxoplasma gondii* is a zoonotic parasite that causes toxoplasmosis in humans and animals. The study results provide baseline data required to educate pig producers, pork handlers and consumers to mitigate toxoplasmosis along the value chain.

**Abstract:** *Toxoplasma gondii* is a ubiquitous protozoan parasite of public health concern, with severe health consequences among immunocompromised individuals and pregnant mothers. Pigs are intermediate hosts of this zoonotic parasite and consumption of undercooked pork is a risk factor for *T. gondii* infection. We investigated the seroprevalence and risk factors for *T. gondii* in pigs in Kenya. A cross-sectional study was conducted at a non-integrated abattoir in Kiambu, Kenya, where 446 blood samples were collected from slaughtered pigs between 5 January and 5 March 2021. For each pig sampled, additional data were collected on the county of origin, farm size, sex and live weight. Serum was harvested from each pig blood sample, and these were subjected to indirect enzyme-linked immunosorbent assay tests to detect the presence of immunoglobulin G (IgG) for *T. gondii* infection. The overall seroprevalence for *T. gondii* was 34.53% (95% C.I. 30.16–39.17). Risk factors for *T. gondii* seropositivity in pigs included farm size ( $p < 0.001$ ) and the live weight of pigs ( $p = 0.044$ ). The high seroprevalence of *T. gondii* indicates that consumers of raw and undercooked pork may be at a high risk of infection. It is therefore recommended that strategies for the mitigation of risk of exposure in populations should prioritize public health education for pig farmers, pork handlers and consumers on risk reduction measures along the pork value chains.

**Keywords:** Kenya; pork; *Toxoplasma gondii*; toxoplasmosis; zoonoses



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## 1. Introduction

*Toxoplasma gondii* is a zoonotic parasite that affects all warm-blooded animals [1]. People get infected by ingesting oocysts in contaminated water, fruits, vegetables, or consumption of tissue cysts in raw or undercooked meat. If infected, people with strong immunity are generally asymptomatic and they would not be aware that they are infected. However, immunocompromised individuals will have serious health complications. These individuals include people living with HIV and AIDS, cancer patients, diabetics, pregnant women, the elderly and very young individuals. Possible health complications associated

with toxoplasmosis include encephalitis, ocular abnormalities, mental disorders and secondary respiratory infections [2]. Transplacental infection in the first trimester of pregnancy leads to miscarriages, stillbirths and congenital anomalies such as hydrocephaly and microcephaly [2]. Pork is an important contributor to the global burden of toxoplasmosis in humans, with toxoplasmosis attributable to pork consumption estimated to result in 2 of the 9 disability-adjusted life years lost per 100,000 population globally per year [3]. Pigs acquire infection postnatally by consuming sporulated oocysts in contaminated feed and water, eating bradyzoites in tissue cysts of infected intermediate hosts and prenatally through the placenta [2]. Pigs infected with *T. gondii* can develop self-limiting fever and inappetence, and rarely, acute toxoplasmosis can result in death, with reports of epizootic toxoplasmosis [4].

A recent global systematic literature review and meta-analysis estimated the global seroprevalence of *T. gondii* in pigs at 19%, with the lowest prevalence region being found in Europe likely due to a combination of climate, production systems and specific control measures. This review identified only nine published papers from Africa over the last 30 years, highlighting the paucity of data on the occurrence of toxoplasmosis and the associated risk practices in pigs within the continent [5]. Due to this paucity of data and listing of toxoplasmosis as a neglected tropical disease, this study was designed to establish baseline data on the presence of *T. gondii* in pigs slaughtered at a local pig abattoir in Kiambu, and associated risk factors. The findings from this study are useful in supporting the formulation of measures to reduce the risk of exposure of workers in animal food processing plants to toxoplasmosis.

## 2. Materials and Methods

### 2.1. Study Site, Design and Sampling Method

The study site and design have been previously explained in [6]. Briefly, this was a cross-sectional study conducted at a non-integrated pig abattoir in Kiambu County, Kenya, with a capacity to slaughter approximately 40 pigs per day, and which supplies approximately 10% of the pork demand of Nairobi [7]. This study was embedded within a larger study requiring 529 pigs to be sampled, but for the determination of *T. gondii* prevalence, a minimum sample size of 384 pigs was calculated based on an expected 50% prevalence, chosen due to a lack of previous studies of *T. gondii* in pigs in Kenya and utilizing the formula for prevalence surveys from [8]. Systematic sampling (every second pig presented for slaughter on each given sampling day) was used to recruit pigs for the study and an informed consent form was obtained and signed by persons who presented the pigs for slaughter. Additional data regarding the pig's county of origin, farm size, sex and liveweight were collected and entered in Open Data Kit (ODK) and uploaded to the International Livestock Research Institute (ILRI) server.

### 2.2. Blood Collection and Processing

At the exsanguination point, blood was collected from the jugular vein of the slaughtered pig into a 10 mL plain vacutainer tube. The collected blood samples were kept in cool boxes packed with icepacks and transported to the laboratory to the Department of Public Health Pharmacology and Toxicology, University of Nairobi, for further processing and testing for the presence of *T. gondii* antibodies. At the laboratory, blood samples were centrifuged at 3000 revolutions per minute (rpm) for 20 min at room temperature to obtain the serum. The serum obtained was separated into two aliquots of 2 mL in cryovials and stored at  $-20^{\circ}\text{C}$  for further analysis. Finally, the serum samples were defrosted in batches and tested in duplicates to detect the presence of *T. gondii*-specific IgG using ID Screen<sup>®</sup> Toxoplasmosis Indirect Multi-species (Idvet, Grabels, France), according to the manufacturer's guidelines.

### 2.3. Serological Testing

Of each serum sample and one each of positive and negative controls, 150  $\mu\text{L}$  was added to 150  $\mu\text{L}$  of the substrate solution in individual Eppendorf tubes, vortexed and incubated at room temperature for 20 min. Afterward, the samples and controls were centrifuged at 1500 rpm for 10 min. Of the supernatant, 150  $\mu\text{L}$  was transferred to an Eppendorf tube with neutralization buffer, then mixed by vortexing to form a sample of  $\frac{1}{4}$  dilution, and 100  $\mu\text{L}$  of the positive and negative controls, and each sample, in duplicate, were added to a 96-well ELISA plate, sealed with a microplate sealer, then incubated for 15 min at 37 °C while shaking at 800 rpm. The wells were washed 5 times with the wash buffer, after which 100  $\mu\text{L}$  conjugate solution was added to each well and incubated for 15 min at 37 °C while shaking at 800 rpm. Washing was repeated five times before 100  $\mu\text{L}$  of substrate solution (TMB) was added in to each well and incubated in the dark for 15 min at room temperature before the reaction was stopped with 50  $\mu\text{L}$  stop solution that was added to each well. The plates were read at 450 nm with a 655 nm filter. Samples that presented a sample/positive percentage (SP%) greater than 50% were considered positive. Commercial ELISA has an estimated sensitivity of 98.9% and specificity of 92.7% [9].

### 2.4. Data Management and Analysis

Data were downloaded from the ILRI server as .csv files, cleaned and merged for statistical analysis in R version 3.6.0 (26 April 2019) [10]. *Toxoplasma gondii* seroprevalence and prevalence of potential risk factors with their 95% confidence interval were calculated using the DescTools package in R [11]. Chi-square ( $\chi^2$ ) test and Student's *t*-test were used to determine the association between *T. gondii* status and independent variables using the Arsenal package in R statistical tool [12].

## 3. Results

The overall seroprevalence of *T. gondii* was 34.53% (95% C.I. 30.16–39.17%). Univariate analysis revealed that the farm size ( $p < 0.001$ ) and live weights of pigs ( $p = 0.044$ ) were factors that were significantly associated with *T. gondii* seropositivity, as shown in Table 1.

**Table 1.** Descriptive statistics for variables and their association with *T. gondii* sero-positivity in pigs slaughtered at a non-integrated abattoir in Kiambu, Kenya.

Categorical Variables								
Variable	Category	Category Frequency (n)	Percent by Category (n/N <sup>1</sup> )	95% C.I. <sup>2</sup>	Number Positive for <i>T. gondii</i> Antibodies	Percentage Positive for <i>T. gondii</i> Antibodies	95% C. I. <sup>3</sup>	<i>p</i> -Values
Sex	Female	243	54.86	50.08–59.54	85	34.98	29.06–41.38	0.744
	Male	200	45.15	40.46–49.92	67	33.50	27.07–40.55	
County of origin of the pig	Nairobi	56	12.81	9.90–16.40	24	42.86	29.97–56.73	0.083
	Kiambu	342	78.26	74.03–81.99	120	35.09	30.08–40.44	
	Makueni	4	0.92	0.29–2.49	1	25.00	1.32–78.06	
	Kajiado	15	3.43	2.00–5.72	3	20.00	5.31–48.44	
	Nakuru	14	3.20	1.83–5.45	1	7.14	0.37–35.83	
	Homabay	4	0.92	0.30–2.50	0	0.00	0.00–60.42	
	Murang'a	2	0.45	0.08–1.82	0	0.00	0.00–60.42	
Husbandry type	Housed	425	97.70	95.67–98.83	146	34.35	29.88–39.11	0.344
	Outdoor	10	2.30	1.17–4.32	2	20.00	3.54–55.78	

Table 1. Cont.

Categorical Variables								
Variable	Category	Category Frequency (n)	Percent by Category (n/N <sup>1</sup> )	95% C.I. <sup>2</sup>	Number Positive for <i>T. gondii</i> Antibodies	Percentage Positive for <i>T. gondii</i> Antibodies	95% C. I. <sup>3</sup>	p-Values
Farm size (number of pigs)	<10	270	62.07	57.31–66.62	110	40.74	34.87–46.88	<0.001 <sup>4</sup>
	>100	45	10.34	7.72–13.69	4	8.89	2.89–22.13	
	10 < 50	114	26.21	22.19–30.65	33	28.95	21.03.38.31	
	50 < 100	6	1.38-	0.56–3.13	1	16.67	0.88–63.52	
Continuous variables								
Variable	Range		Mean		SD <sup>5</sup>			p-value
Live weight	13–230		58.87		25.54			p = 0.044 <sup>6</sup>

These independent variables were strongly correlated ( $p = 0.0012$ ). Therefore, no further analysis was performed as the assumption of no multicollinearity between explanatory variables would have been breached in a logistic regression model. <sup>1</sup> Due to the rapid nature of the slaughter process, we were not able to collect all the data for every pig; hence, N varies per variable; <sup>2</sup> Confidence interval; <sup>3</sup> Confidence interval; <sup>4</sup> Highly significant association; <sup>5</sup> Standard deviation (SD); <sup>6</sup> Statistically significant association.

#### 4. Discussion

This study reported a *T. gondii* IgG seroprevalence of 34.53% in pigs presented for slaughter in an abattoir that supplies unprocessed pork to Nairobi and peri-urban areas. The high seropositivity indicates parasite exposure in pigs raised for slaughter in this pork value chain of Nairobi and suggests a potential zoonotic risk for consumers of this pork. Our findings are comparable with results of the sero-prevalence of anti *T. gondii* IgG in pigs, previously reported at 46.2% in Nigeria [13] and 32.1% in central Ethiopia [14].

In this study, liveweight was used as a proxy for the age of the pigs [15], and there was a significant association between live weight and *T. gondii* sero-positivity ( $p = 0.04$ ). This suggests that exposure to the parasite is likely to increase with the length of time animals are reared in the farm environment and where they are likely to be in contact with infective oocysts. This argument is consistent with findings from [16,17], who reported that an increase in age was associated with *T. gondii* seroprevalence. Farm size was also a significant risk factor ( $p < 0.001$ ), with the prevalence of infection decreasing in farms with over 100 animals. Similar results have been reported by [18], where sows from small farm sizes keeping less than 29 sows were 4.5 times more likely to be seropositive for *T. gondii* when compared with those from large farm sizes keeping more than 29 sows, and [19] who reported that *T. gondii* seropositivity decreased in pig farms with more than 50 animals. It can be argued that farms with larger herd sizes may have better husbandry practices, including proper hygiene practices, rodent control systems, supply of clean water and uncontaminated feed, which play a role in reducing the exposure at infective stages of the parasite within the farm environment. Further investigations of on-farm practices predisposing pigs to infection with *T. gondii*, such as the presence of cats and appropriate biosecurity practices, are proposed in order to make contextually appropriate recommendations to farmers.

The demand for pork in Kenya has been anticipated to rise at a rate of 400 tonnes yearly with the population rise of approximately 1 million persons and eating rate of 0.4 kg per person [20]. The increasing demand for pork suggests that despite overtime, there may be an increased risk of exposure to *T. gondii* through pork consumption if mitigation measures are not put in place. This study has demonstrated that slaughtered pigs have evidence of infection with *T. gondii*, and that small farm sizes and increased liveweight are important risk factors for seropositivity. Mitigating activities are needed in this value chain, including improved farm biosecurity and public health education for consumers, particularly those in high-risk categories.

## 5. Conclusions

This study has demonstrated high seropositivity of *T. gondii* in pigs slaughtered for human consumption in Nairobi. Therefore, pork consumers, especially those who consume raw and undercooked pork, may be at risk of infection. The seroprevalence may be an indication of the presence of infective stages of *T. gondii* either in the environment, pig premises, water or feed. The decreasing prevalence in farms with more than 100 animals may suggest that biosecurity and hygiene practices may be enhanced in these farms, but there is a need for further research on the exposure of pigs to *T. gondii* at the farm level in Kenya to gain insights into the contextual risk factors and design appropriate mitigation strategies.

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**Institutional Review Board Statement:** This study was approved by the International Livestock Research Institute, Institutional Animal Care and Use Committee, (ILRI IACUC ref. no. 2019-36) and the Institutional Research Ethics Committee (ILRI-IREC 2020-14). An additional permit was obtained from the National Commission for Science, Technology, and Innovation (NACOSTI/P/20/4847). Both the National and County Directorate of Veterinary Services granted permission to conduct this research in the study area.

**Informed Consent Statement:** Informed consent statement was obtained, and the form was signed by persons who presented the pigs for slaughter.

**Data Availability Statement:** All data are freely available at: <https://doi.org/10.17638/datacat.liverpool.ac.uk/1441>.

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