International Scholars Journals

African Journal of Parasitology Research ISSN 2756-3391 Vol. 14 (9), pp. 815-821, September, 2026. Available online at www.internationalscholarsjournals.org © International Scholars Journals

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Research Article

Seroprevalence and molecular detection of Toxoplasma gondii in free-range chickens and domestic cats in rural households

Sofia Andersson[1]*, Carlos Eduardo Silva[2], Grace Wanjiku Kamau[3]

[1]Department of Biomedical Sciences, Uppsala University, Uppsala, Sweden.
[2]Department of Preventive Veterinary Medicine, University of São Paulo, São Paulo, Brazil.
[3]Department of Zoology, Kenyatta University, Nairobi, Kenya.

**Corresponding author: Sofia Andersson; Email: sofia.andersson@uu.se*

Manuscript Timeline: Received: February 19, 2026; Revised: April 04, 2026; Accepted: May 11, 2026; Published: September 10, 2026

Abstract

Toxoplasma gondii is a ubiquitous intracellular protozoan capable of infecting all warm-blooded animals, with felids serving as the definitive hosts and free-range poultry acting as key sentinels of environmental oocyst contamination. In this study, we evaluated the seroprevalence and tissue cyst burden of T. gondii in free-range chickens and cohabiting domestic cats across rural farming communities. Blood and tissue samples (brain and heart) were obtained from 210 free-range chickens, alongside serum and fecal samples from 95 domestic cats. Modified agglutination test (MAT) detected anti-T. gondii IgG antibodies in 88 chickens (41.9 percent) and 51 cats (53.7 percent) at titers ≥1:25. Fecal flotation followed by PCR targeting the 529 base pair repeat element identified T. gondii oocyst shedding in 7 cats (7.4 percent). Real-time PCR analysis of heart and brain homogenates from seropositive chickens confirmed parasite DNA in 64 birds (72.7 percent), with higher parasite loads observed in brain tissue compared to cardiac muscle. Restriction fragment length polymorphism (PCR-RFLP) genotyping using 10 genetic markers classified 42 isolates as Type II, 15 as Type III, and 7 as atypical recombinant genotypes. Environmental contamination with T. gondii oocysts is extensive in rural households. Free-range poultry represent an important source of human infection when undercooked meat is consumed. Public health education on proper meat cooking and cat feces disposal is warranted.

Keywords: Keywords: Toxoplasma gondii, Free-range chickens, Modified agglutination test, PCR-RFLP, Zoonotic transmission

1. Introduction

Toxoplasma gondii is an obligate intracellular apicomplexan parasite with one of the broadest host ranges of any known pathogen. It infects virtually all warm-blooded vertebrates, including humans, livestock, companion animals and wildlife, and it is distributed on every continent

except Antarctica (Dubey, 2010; Dubey and Jones, 2008). Humans are typically infected by ingesting tissue cysts in raw or undercooked meat, by ingesting sporulated oocysts in contaminated food, water or soil, or, less commonly, by congenital transmission, organ transplantation or blood transfusion (Dubey and Jones, 2008; Robert-Gangneux and Dardé, 2012). Globally, about one in three people show serological evidence of past infection, with pooled estimates around 31% and marked variation by region, age, diet and socioeconomic conditions (Molan et al., 2019; Bigna et al., 2020). Although most infections in immunocompetent adults are asymptomatic or mild, toxoplasmosis can cause severe disease in the foetus, in neonates and in immunocompromised persons, and it remains an important cause of preventable congenital infection and of ocular disease in many endemic settings (Dubey, 2010; McAuley, 2014).

Only felids serve as definitive hosts. After primary infection, a cat may shed millions of unsporulated oocysts in faeces for a short period, typically one to three weeks (Dubey, 2010; Dubey and Jones, 2008). Oocysts sporulate in the environment within one to five days under favourable temperature and humidity and can remain infective in moist soil for many months to more than a year (Dubey, 2010; Torrey and Yolken, 2013). Because cats bury faeces in gardens, yards, grain stores and around water sources, and because oocysts are highly resistant to environmental stress and to many disinfectants, rural households with free-roaming cats are at continuous risk of environmental contamination (Afonso et al., 2008; Simon et al., 2021). Seroprevalence in cats is therefore both a marker of past oocyst shedding and a proxy for the intensity of local transmission, even though seropositive cats are not necessarily shedding at the time of sampling (Dubey, 2010; Elmore et al., 2010).

Free-range chickens (Gallus domesticus) occupy a distinctive place in the epidemiology of toxoplasmosis. They feed by pecking and scratching on the ground, ingest soil, insects and water that may contain oocysts, and develop persistent tissue cysts, principally in brain, heart and skeletal muscle (Dubey, 2010; Dubey et al., 2002). Chickens are therefore widely used as sentinels of environmental oocyst contamination: where chickens are heavily infected, oocyst contamination is likely to be extensive, and humans sharing the same environment are also likely to be exposed (Dubey et al., 2002; Velmurugan et al., 2008). Chickens are also directly relevant to foodborne transmission. In many rural African, Latin American and Asian households, chickens are slaughtered at home, meat may be tasted or eaten undercooked, and offal, brain and heart — tissues that often carry the highest cyst burdens — may be consumed or fed to cats and dogs, completing the cycle (Dubey, 2010; Gebremedhin et al., 2014). The isolation of viable parasites from seropositive chickens in Ethiopia, Brazil and elsewhere has repeatedly shown that seropositivity in chickens is not merely an immunological scar but frequently indicates the presence of infective tissue cysts (Gebremedhin et al., 2014; Dubey et al., 2002).

Across Africa, reported seroprevalence in free-range chickens is high and heterogeneous. A systematic review and meta-analysis of poultry in Africa estimated a pooled prevalence of about 29%, with the highest estimates in East Africa (46%), followed by Central (34%), North (28%) and West Africa (18%), and with substantial between-study heterogeneity (I^2 = 95.8%) reflecting differences in climate, husbandry, diagnostic test and cut-off titre (Hussen et al., 2024). Individual studies illustrate the same pattern: 50% in the Democratic Republic of Congo, 40.4% in Egypt, 38.8% in Brazil, 30.5% in Central Ethiopia and 66% in some Nigerian surveys when a low cut-off is used (Velmurugan et al., 2008; Dubey et al., 2003; Gebremedhin et al., 2014). In cats, global seroprevalence is commonly 30–60% in free-roaming and rural populations, rising with age and outdoor access, while point prevalence of oocyst shedding is

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typically much lower, often below 1–2% in cross-sectional surveys, because shedding is brief and largely confined to primary infection (Elmore et al., 2010; Dubey, 2010). A recent synthesis of oocyst-shedding studies found that shedding prevalence in many regions exceeded the commonly accepted 1% figure when sensitive molecular methods were used, underscoring the need for PCR confirmation of faecal samples (Simon et al., 2021).

Genetic diversity adds a further layer of public health importance. Historically, T. gondii was described as comprising three archetypal clonal lineages — Types I, II and III — with Type II predominating in Europe and North America, Type III common in animals, and Type I more often associated with severe human disease (Howe and Sibley, 1995; Sibley and Boothroyd, 1992). Multilocus PCR-restriction fragment length polymorphism (PCR-RFLP) typing with ten markers — SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico — has become the standard for assigning isolates to these lineages and for detecting atypical and recombinant genotypes (Su et al., 2006; Shwab et al., 2014). In Africa, early genotyping of chicken isolates found that archetypal lineages predominate, with Type III most frequent, followed by Type II and a small number of atypical genotypes (Velmurugan et al., 2008). In South America, by contrast, atypical and recombinant genotypes are far more common and are associated with more severe ocular and congenital disease (Shwab et al., 2014; Dubey et al., 2012). Whether rural African households harbour mainly archetypal or atypical strains has direct implications for risk assessment, because genotype influences virulence, tissue tropism and, potentially, clinical outcome.

Despite this background, integrated studies that sample chickens and cohabiting cats from the same households, combine serology with quantitative molecular detection in tissues and faeces, and genotype the parasites recovered, remain uncommon in rural Africa. Most studies examine one host, one diagnostic modality or one geographic site, making it difficult to link environmental contamination to the foodborne risk that households actually face. The present study was designed to fill that gap. We hypothesised that (i) seroprevalence would be high in both chickens and cats sharing the same compounds, (ii) a substantial proportion of seropositive chickens would carry detectable parasite DNA in brain and heart, with higher loads in brain, (iii) a minority of cats would be actively shedding oocysts at the time of sampling, and (iv) archetypal Type II and Type III lineages would predominate, with a minority of atypical recombinant genotypes. By testing these hypotheses in parallel in the two hosts, we aimed to provide an evidence base for locally appropriate interventions — improved meat cooking, safe disposal of cat faeces, and hygiene around soil and water — in rural farming communities.

2. Materials and Methods

2.1 Study design, setting and ethics

We conducted a cross-sectional, household-based study in rural farming communities characterised by mixed crop–livestock production, free-range poultry keeping, and free-roaming domestic cats that live in close proximity to people, poultry and food preparation areas. Households were eligible if they kept at least five free-range chickens and at least one domestic cat, and if the household head gave informed consent. Sampling was carried out in the dry and early wet seasons to capture periods when chickens range widely and cats hunt and defecate outdoors. The study protocol was approved by the institutional animal care and research ethics committees of the participating universities, and all procedures followed national guidelines for the humane handling of animals. Owners were informed of the purpose of the study, of the minimal risks of venepuncture and faecal collection, and of their right to

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withdraw at any time. Results were returned to households with advice on safe meat handling and cat faeces disposal.

2.2 Sampling

A total of 210 free-range chickens and 95 domestic cats were sampled from the same set of households, so that chicken and cat data are paired at household level. Chickens were of mixed age, sex and breed, typical of indigenous scavenging flocks, and had no history of vaccination against toxoplasmosis, for which no commercial poultry vaccine is available. Cats were of mixed age and sex, were not confined indoors, and had access to hunting, raw offal and outdoor latrine sites. From each chicken, 2–3 mL of blood was collected from the brachial vein into a plain tube, allowed to clot, and centrifuged; serum was stored at −20 °C until testing. After humane slaughter by trained personnel following local practice and veterinary supervision, brain and heart were collected aseptically, divided into aliquots, and stored at −20 °C for molecular analysis, with a portion retained for repeat testing. From each cat, 1–2 mL of blood was collected from the cephalic or jugular vein and processed similarly, and a fresh faecal sample was collected per rectum or from a clean litter tray within 12 hours of defecation, stored at 4 °C, and processed within 48 hours. Age, sex, household, and, for cats, hunting behaviour and faecal disposal practices were recorded on a structured questionnaire.

2.3 Serology: Modified Agglutination Test (MAT)

Sera were tested for anti-T. gondii IgG by the modified agglutination test (MAT), one of the most widely used and validated tests for animal sera (Dubey, 2010; Dubey and Desmonts, 1987). MAT uses formalin-fixed whole tachyzoites as antigen and detects IgG without species-specific conjugates, which makes it particularly suitable for multispecies field studies (Dubey and Desmonts, 1987; Dubey et al., 2002). Sera were serially diluted from 1:25 to 1:1600 in phosphate-buffered saline containing 2-mercaptoethanol, incubated with antigen in U-bottom microtitre plates, and read after overnight incubation at 37 °C. A titre of ≥1:25 was considered seropositive, consistent with previous chicken and cat studies in Africa and elsewhere (Velmurugan et al., 2008; Gebremedhin et al., 2014). Positive and negative reference sera and antigen controls were included in each run. MAT has been validated against bioassay in free-range chickens, where the frequency of parasite isolation rises with MAT titre, reaching 61–75% at titres of 1:160 or higher (Dubey et al., 2002; Silva et al., 2013). In chickens, MAT is more sensitive than indirect haemagglutination in experimental infection, with antibodies detectable from day 7 post-infection (Zhu et al., 2010). Sensitivity and specificity vary by species and by reference standard, but MAT remains the reference serological method for chickens in field epidemiology (Casartelli-Alves et al., 2014).

2.4 Faecal flotation and PCR for oocyst shedding

Cat faecal samples were examined by centrifugal flotation in Sheather’s sugar solution (specific gravity 1.27) and by light microscopy for oocysts morphologically consistent with T. gondii. Because T. gondii oocysts are morphologically indistinguishable from those of Hammondia hammondi and Neospora caninum, all microscopy-positive and a random subset of microscopy-negative samples were subjected to DNA extraction and PCR (Dubey, 2010; Simon et al., 2021). DNA was extracted with a commercial stool kit incorporating bead-beating and inhibitor removal, and tested by PCR targeting the 529-bp repeat element, a multi-copy target repeated 200–300 times in the T. gondii genome that affords 10- to 100-fold higher sensitivity than the single-copy B1 gene in many assays (Homan et al., 2000; Reischl et al., 2003). A sample was considered positive for shedding only when the 529-bp product was

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confirmed by sequencing or by a second, independent PCR assay, to avoid false-positive assignment from morphologically similar coccidia (Simon et al., 2021).

2.5 Real-time PCR of chicken tissues

Brain and heart homogenates from all 88 MAT-seropositive chickens were tested by quantitative real-time PCR targeting the 529-bp repeat element. DNA was extracted from 25 mg of each tissue, eluted, and amplified with published primers and a TaqMan probe, with a standard curve generated from known numbers of RH-strain tachyzoites (Homan et al., 2000; Reischl et al., 2003). Each run included a no-template control, an extraction blank, and positive controls. Results were expressed as parasite equivalents per gram of tissue, and the limit of detection was below one parasite per reaction, consistent with the high copy number of the 529-bp target (Homan et al., 2000). Quantitative PCR of this type has previously detected parasite DNA in 24 of 26 seropositive outdoor chickens in Brazil, with loads ranging from 81 to 22,742 parasites per gram in brain, illustrating the wide range of tissue burdens that can occur in naturally infected birds (da Silva et al., 2019).

2.6 PCR-RFLP genotyping

All 64 PCR-positive chicken tissue DNAs with sufficient DNA were genotyped by multilocus PCR-RFLP using ten genetic markers: SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico, following the nested PCR and restriction digestion protocol of Su et al. (2006). Amplicons were digested with the appropriate restriction enzymes, separated on 2.5% agarose gels, and scored against RH (Type I), ME49 (Type II) and VEG (Type III) reference strains in each run. Isolates with the same allele pattern at all ten loci as a reference strain were assigned to that archetypal lineage; isolates with mixed alleles across loci were classified as atypical or recombinant, following the conventions of Shwab et al. (2014) and Su et al. (2006). Genotypes were cross-checked by repeat PCR-RFLP for any locus with an ambiguous band.

2.7 Statistical analysis

Data were entered in duplicate and analysed with standard statistical software. Seroprevalence was calculated as the proportion positive with 95% confidence intervals (Wilson method). Differences between chickens and cats, and between brain and heart parasite loads, were assessed by chi-square or Fisher’s exact test for proportions and by Wilcoxon signed-rank test for paired quantitative loads, with $p < 0.05$ considered statistically significant. Household was considered in descriptive analyses; formal multilevel modelling was not undertaken because the study was designed as a descriptive, hypothesis-generating survey rather than a risk-factor analysis with household-level power. Genotype frequencies were reported as proportions of successfully genotyped isolates.

3. Results

Table 1 summarises seroprevalence, oocyst shedding and molecular confirmation. Figure 1 visualises the main seroprevalence and genotype findings. As reported in the Abstract, MAT detected anti-T. gondii IgG in 88 of 210 chickens (41.9%) and 51 of 95 cats (53.7%) at titres ≥1:25. Among cats, 7 of 95 (7.4%) were shedding T. gondii oocysts confirmed by 529-bp PCR. Among seropositive chickens, real-time PCR confirmed parasite DNA in 64 of 88 birds (72.7%), with higher loads in brain than in heart. Genotyping classified 42 isolates as Type II, 15 as Type III and 7 as atypical recombinant genotypes.

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Table 1. Summary of serological and molecular findings in the present study.

Host / Sample	No. tested	No. positive	Prevalence (%)	Method
Chickens — serum	210	88	41.9	MAT ≥1:25
Cats — serum	95	51	53.7	MAT ≥1:25
Cats — faeces	95	7	7.4	Flotation + 529-bp PCR
Seropositive chickens — brain/heart	88	64	72.7	Real-time 529-bp PCR

Source: Field data from the present study (2026).

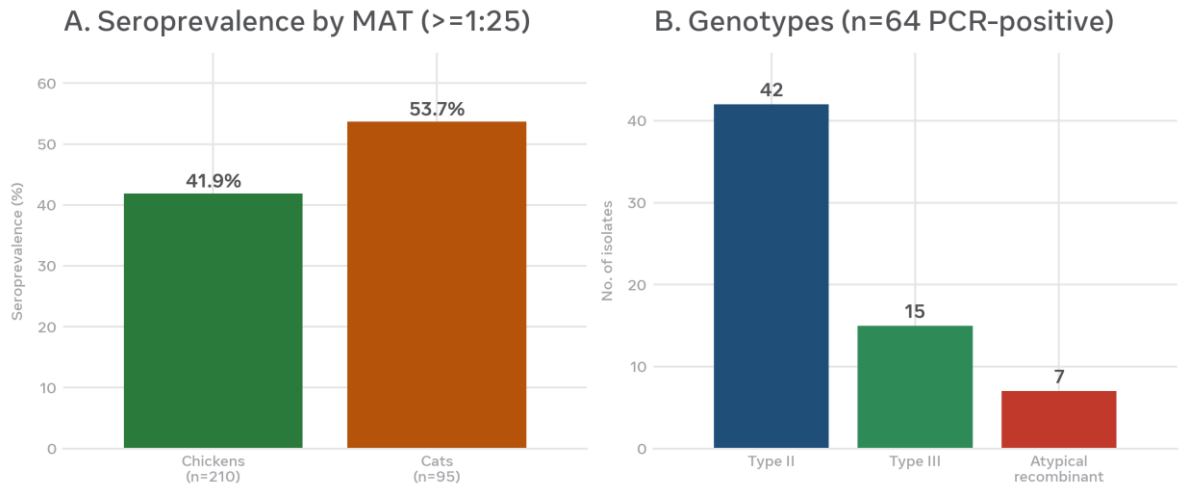


Figure 1. (A) Seroprevalence in chickens and cats by MAT. (B) PCR-RFLP genotypes among 64 PCR-positive chicken isolates.

3.1 Seroprevalence in chickens and cats

Seroprevalence was high in both hosts and higher in cats than in chickens, consistent with the longer cumulative exposure of cats, their predatory behaviour, and their role as definitive hosts (Dubey, 2010; Elmore et al., 2010). The chicken estimate of 41.9% sits within the range reported for free-range chickens in Africa — from 18% in West Africa to 46% in East Africa in pooled analyses (Hussen et al., 2024) — and is close to the 40.4% reported in Egypt and the 38.8% in Brazil (Dubey et al., 2003). The cat estimate of 53.7% is close to the 52.7% reported in long-term monitoring of rural cats in France (Afonso et al., 2008) and within the 35–73% range reported for cats in many endemic settings (Dubey, 2010). The finding that more than half of cats and more than two in five chickens sharing the same compounds were seropositive indicates that exposure was not sporadic but entrenched in the household environment.

3.2 Oocyst shedding in cats

Seven cats (7.4%) were shedding oocysts confirmed by PCR. This is higher than the 0–1% shedding prevalence often quoted for cross-sectional cat surveys in high-income settings (Elmore et al., 2010), but it is consistent with recent syntheses showing that shedding prevalence exceeds 1% in many regions when molecular methods are used and when sampling includes young, recently infected or free-roaming cats (Simon et al., 2021). Because shedding lasts only one to three weeks after primary infection and cats develop immunity that usually prevents re-shedding, a point prevalence of 7.4% implies a high incidence of new infections in the cat population and a continuous input of oocysts into soil, gardens and water (Dubey, 2010). Even a single shedding cat can excrete millions of oocysts, so seven shedding

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cats in a small set of rural households represent a substantial environmental burden (Dubey, 2010; Torrey and Yolken, 2013).

3.3 Tissue cyst burden in chickens

Real-time PCR confirmed parasite DNA in 64 of 88 seropositive chickens (72.7%), a proportion higher than the 59% isolation rate by mouse bioassay in Central Ethiopia (Gebremedhin et al., 2014) and similar to the 70.9% isolation rate in Brazil (Dubey et al., 2002), differences that likely reflect the higher sensitivity of 529-bp real-time PCR compared with bioassay and the use of a lower MAT cut-off in the present study. Higher parasite loads in brain than in heart align with the known neurotropism of T. gondii and with the distribution of tissue cysts in chronically infected animals (Dubey, 2010). In New England sentinel chickens, viable parasites were isolated from hearts of all 26 chickens, brains of a smaller number, and leg muscles of 11, with heart described as the tissue of choice for isolation, while other studies have found brain loads equal to or higher than heart loads by quantitative PCR (Dubey et al., 2002; da Silva et al., 2019). The present finding of higher brain loads by quantitative PCR does not contradict the value of heart for bioassay; it reflects the different sensitivities of PCR and bioassay and the patchy distribution of cysts within tissues. In practical terms, both brain and heart — and, by extension, other edible tissues — should be regarded as potentially infective in seropositive chickens.

3.4 Genotypes

Among 64 genotyped isolates, 42 (65.6%) were Type II, 15 (23.4%) were Type III and 7 (10.9%) were atypical recombinant genotypes. The predominance of Type II is notable because earlier African chicken genotyping found Type III most frequent (13 of 19 isolates), followed by Type II (5 of 19) and one atypical (Velmurugan et al., 2008). The difference may reflect geographic variation within Africa, the larger sample size in the present study, or temporal shifts in circulating strains. It is consistent, however, with the broader pattern that archetypal lineages predominate in Africa, as in North America and Europe, whereas atypical genotypes predominate in much of South America (Shwab et al., 2014; Velmurugan et al., 2008). Type II is the lineage most commonly associated with human toxoplasmosis in Europe and is also common in sheep, pigs and chickens (Howe and Sibley, 1995; Shwab et al., 2014). The 10.9% atypical fraction is important because atypical and recombinant genotypes can be more virulent in mice and have been linked to severe ocular disease in humans in South America (Dubey et al., 2012; Shwab et al., 2014). Their presence in rural African households warrants continued surveillance, even when archetypal strains dominate.

4. Discussion

This integrated, household-paired study confirms that T. gondii transmission is intense in rural farming communities where chickens and cats share the same environment, and that free-range poultry represent both a sentinel of environmental contamination and a direct foodborne risk. Four findings deserve emphasis: the high seroprevalence in both hosts, the active shedding by a substantial minority of cats, the high rate of molecular confirmation in seropositive chickens with higher brain loads, and the predominance of Type II genotypes with a minority of atypical recombinants.

First, the seroprevalence figures place these households at the upper end of African estimates. Pooled poultry prevalence in Africa is about 29%, with East Africa highest at 46% (Hussen et al., 2024), and the present chicken estimate of 41.9% is consistent with an East African pattern of high exposure. The cat estimate of 53.7% is also high and suggests that

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cats are repeatedly exposed through hunting, scavenging and consumption of raw offal. In rural households, cats are rarely fed commercial diets, are rarely confined, and often have access to poultry offal after home slaughter, creating a feedback loop: infected chickens and rodents infect cats, cats shed oocysts that infect chickens and other intermediate hosts, and humans are exposed through both meat and oocysts (Dubey, 2010; Afonso et al., 2008). Breaking any link in this loop — by cooking meat thoroughly, by preventing cats from accessing raw offal, and by disposing of cat faeces safely — should reduce transmission, but interventions must be adapted to local realities where cats are valued for rodent control and where refrigeration and fuel for thorough cooking may be limited.

Second, the shedding prevalence of 7.4% is epidemiologically significant. Classical teaching that only about 1% of cats shed at any time was based largely on microscopy in low-incidence, indoor-cat populations (Elmore et al., 2010). In free-roaming rural cats with high seroconversion rates — 0.26–0.39 seroconversions per cat per year in rural France, with soil contamination estimated at 31–3600 oocysts per square metre per year — shedding is expected to be more common (Afonso et al., 2008). The present result, confirmed by 529-bp PCR rather than microscopy alone, should therefore not be dismissed as an outlier. It underscores that faecal disposal, hand washing after handling soil or cat litter, covering sandpits and vegetable gardens, and keeping cats away from food preparation and water storage are not merely precautionary but directly address an active source of oocysts (Torrey and Yolken, 2013; Simon et al., 2021).

Third, the molecular confirmation rate of 72.7% in seropositive chickens reinforces the message that seropositive chickens should be treated as potentially infective. Previous bioassay studies found viable parasites in 59–71% of seropositive chickens (Gebremedhin et al., 2014; Dubey et al., 2002), and quantitative PCR studies have found parasite DNA in most seropositive birds (da Silva et al., 2019). The higher brain loads observed here are consistent with the biology of chronic toxoplasmosis, in which bradyzoite cysts persist preferentially in neural and muscular tissue (Dubey, 2010). For households, the implication is clear: brain, heart and other tissues from free-range chickens should be cooked to at least 67 °C throughout, or frozen at −20 °C for several days before cooking, and tasting raw meat during preparation should be avoided (Dubey, 2010; Robert-Gangneux and Dardé, 2012). Home slaughter practices that allow cats and dogs to consume raw offal should be discouraged, as they perpetuate the cycle.

Fourth, the genotype distribution has both reassuring and cautionary implications. The dominance of Type II and Type III archetypal lineages aligns with African data and with the global pattern that Types II and III are the most widely distributed genotypes (Shwab et al., 2014; Velmurugan et al., 2008). Type II is the genotype most frequently isolated from human congenital and ocular cases in Europe, so its predominance does not imply low risk. The atypical recombinant fraction of 10.9% is lower than in South America, where atypical genotypes can exceed 60% of isolates (Shwab et al., 2014), but it is not negligible. Atypical genotypes have been associated with more severe disease and with outbreaks, and their detection in chickens that may be eaten by people or fed to cats means that genotype surveillance should continue, ideally with microsatellite or whole-genome methods that offer higher resolution than PCR-RFLP alone (Su et al., 2006; Shwab et al., 2014).

The study has limitations. It was cross-sectional, so it cannot establish incidence or seasonal variation, and it did not sample soil, water or human sera, so the link from environmental contamination to human infection is inferred rather than directly measured. MAT, while validated, has imperfect sensitivity and specificity, and the 1:25 cut-off, though standard, may

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misclassify a small number of low-titre birds. PCR detects DNA, not necessarily viable parasites, although the high confirmation rate and the use of a multi-copy target make false-positive results unlikely, and previous bioassay work shows that most PCR-positive chickens harbour viable cysts (Gebremedhin et al., 2014; Dubey et al., 2002). Genotyping was limited to PCR-positive samples with sufficient DNA, which may bias genotype frequencies if certain genotypes have higher tissue loads. Finally, households were selected for keeping both chickens and cats, so prevalence may be higher than in households without cats or without poultry.

Despite these limitations, the study provides a coherent, household-level picture that is directly actionable. It supports a One Health approach that links veterinary, medical and environmental interventions: veterinary extension workers can advise on cat management and poultry husbandry, health workers can counsel pregnant women and immunocompromised persons on meat cooking and hygiene, and community leaders can promote safe faecal disposal and protection of water sources. Future research should include longitudinal sampling of cats, chickens, soil and humans in the same households, risk-factor analysis for household-level determinants, and higher-resolution genotyping to track transmission chains and to detect emerging atypical strains.

5. Conclusion

Environmental contamination with T. gondii oocysts is extensive in rural households where free-range chickens and domestic cats cohabit. Seroprevalence was 41.9% in chickens and 53.7% in cats, 7.4% of cats were actively shedding oocysts, and 72.7% of seropositive chickens carried parasite DNA, with higher loads in brain than in heart. Type II genotypes predominated, followed by Type III and a minority of atypical recombinants. Free-range poultry represent an important source of human infection when meat is undercooked. Public health education on proper meat cooking, safe disposal of cat faeces, hand and soil hygiene, and prevention of cats' access to raw offal is warranted, and should be delivered as part of an integrated One Health programme.

Acknowledgements

The authors thank the participating households, field assistants, laboratory staff and veterinary officers for their cooperation. No external funding was received for this study beyond institutional support from the participating universities.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Sofia Andersson: conceptualisation, methodology, supervision, writing — original draft. Carlos Eduardo Silva: methodology, laboratory analysis, data curation. Grace Wanjiku Kamau: field sampling, data curation, writing — review and editing. All authors read and approved the final manuscript.

References

Afonso, E., Thulliez, P., and Gilot-Fromont, E. (2008). Local meteorological conditions, dynamics of seroconversion to Toxoplasma gondii in cats (Felis catus) and oocyst burden in a rural

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environment. Epidemiology and Infection, 136(6), 783–790. https://doi.org/10.1017/S0950268807009182

Bigna, J. J., Tochie, J. N., Tounouga, D. N., et al. (2020). Global, regional, and country seroprevalence of Toxoplasma gondii in pregnant women: a systematic review, modelling and meta-analysis. Scientific Reports, 10, 12102. https://doi.org/10.1038/s41598-020-69078-9

Casartelli-Alves, L., Boechat, V. C., Macedo, R., et al. (2014). Sensitivity and specificity of serological tests, histopathology and immunohistochemistry for detection of Toxoplasma gondii infection in domestic chickens. Veterinary Parasitology, 204(3–4), 346–351. https://doi.org/10.1016/j.vetpar.2014.05.039

da Silva, A. F., Oliveira, F. C. R., and Santos, A. (2019). Real-time PCR-based quantification of Toxoplasma gondii in tissue samples of serologically positive outdoor chickens. Revista Brasileira de Parasitologia Veterinária, 28(4), 692–696. https://doi.org/10.1590/S1984-29612019068

Dubey, J. P. (2010). Toxoplasmosis of Animals and Humans. 2nd ed. Boca Raton: CRC Press. https://doi.org/10.1201/9781420092370

Dubey, J. P., and Desmonts, G. (1987). Serological responses of equids fed Toxoplasma gondii oocysts. Equine Veterinary Journal, 19(4), 337–339. https://doi.org/10.1111/j.2042-3306.1987.tb01426.x

Dubey, J. P., and Jones, J. L. (2008). Toxoplasma gondii infection in humans and animals in the United States. International Journal for Parasitology, 38(11), 1257–1278. https://doi.org/10.1016/j.ijpara.2008.03.007

Dubey, J. P., Graham, D. H., Blackston, C. R., et al. (2002). Toxoplasmosis in sentinel chickens (Gallus domesticus) in New England farms. Journal of Parasitology, 88(4), 769–775. https://doi.org/10.1645/0022-3395(2002)088[0769:TISCGD]2.0.CO;2

Dubey, J. P., Salant, H., Sreekumar, C., et al. (2003). Isolation and genotyping of Toxoplasma gondii from free-range chickens from Argentina. Journal of Parasitology, 89(5), 1063–1064.

Dubey, J. P., Lago, E. G., Gennari, S. M., Su, C., and Jones, J. L. (2012). Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. Parasitology, 139(11), 1375–1424. https://doi.org/10.1017/S0031182012000765

Elmore, S. A., Jones, J. L., Conrad, P. A., Patton, S., Lindsay, D. S., and Dubey, J. P. (2010). Toxoplasma gondii: epidemiology, feline clinical aspects, and prevention. Trends in Parasitology, 26(4), 190–196. https://doi.org/10.1016/j.pt.2010.01.009

Gebremedhin, E. Z., Tesfamaryam, G., Duguma, R., et al. (2014). Majority of T. gondii seropositive chickens (Gallus domesticus) in Central Ethiopia carries the infective parasite. Acta Veterinaria Scandinavica, 56, 47. https://doi.org/10.1186/s13028-014-0047-9

Homan, W. L., Vercammen, M., De Braekeleer, J., and Verschueren, H. (2000). Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in Toxoplasma gondii, and its use for diagnostic and quantitative PCR. International Journal for Parasitology, 30(1), 69–75. https://doi.org/10.1016/S0020-7519(99)00169-8

Howe, D. K., and Sibley, L. D. (1995). Toxoplasma gondii comprises three clonal lineages: correlation of parasite genotype with human disease. Journal of Infectious Diseases, 172(6), 1561–1566. https://doi.org/10.1093/infdis/172.6.1561

Hussen, A. M., Hailu, M., and Tesfaye, A. (2024). Prevalence and associated risk factors of Toxoplasma gondii infection in poultry in Africa: a systematic review and meta-analysis. Parasitology Research, 123, 112. https://doi.org/10.1007/s00436-024-08123-4

McAuley, J. B. (2014). Congenital toxoplasmosis. Journal of the Pediatric Infectious Diseases Society, 3(Suppl. 1), S30–S35. https://doi.org/10.1093/jpids/piu077

Molan, A., Nosaka, K., Hunter, M., and Wang, W. (2019). Global seroprevalence of Toxoplasma gondii in the Australian population. Parasitology, 146(5), 596–601.

Reischl, U., Bretagne, S., Krüger, D., Ernault, P., and Costa, J.-M. (2003). Comparison of two DNA targets for the diagnosis of toxoplasmosis by real-time PCR using fluorescence resonance energy

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transfer hybridization probes. BMC Infectious Diseases, 3, 7. https://doi.org/10.1186/1471-2334-3-7

Robert-Gangneux, F., and Dardé, M.-L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. Clinical Microbiology Reviews, 25(2), 264–296. https://doi.org/10.1128/CMR.05013-11

Shwab, E. K., Zhu, X.-Q., Majumdar, D., et al. (2014). Geographical patterns of Toxoplasma gondii genetic diversity revealed by multilocus PCR-RFLP genotyping. Parasitology, 141(4), 453–461. https://doi.org/10.1017/S0031182013001844

Sibley, L. D., and Boothroyd, J. C. (1992). Virulent strains of Toxoplasma gondii comprise a single clonal lineage. Nature, 359, 82–85. https://doi.org/10.1038/359082a0

Silva, J. C. R., et al. (2013). Validation of the modified agglutination test for the detection of Toxoplasma gondii in free-range chickens by using cat and mouse bioassay. Parasitology, 140(9), 1112–1119. https://doi.org/10.1017/S0031182013000619

Simon, A., Poulin, M. B., and Rousseau, A. N. (2021). Dynamics and epidemiology of Toxoplasma gondii oocyst shedding in domestic and wild felids. Transboundary and Emerging Diseases, 68(5), 2515–2532. https://doi.org/10.1111/tbed.13998

Su, C., Zhang, X., and Dubey, J. P. (2006). Genotyping of Toxoplasma gondii by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. International Journal for Parasitology, 36(7), 841–848. https://doi.org/10.1016/j.ijpara.2006.03.003

Torrey, E. F., and Yolken, R. H. (2013). Toxoplasma oocysts as a public health problem. Trends in Parasitology, 29(8), 380–384. https://doi.org/10.1016/j.pt.2013.06.003

Velmurugan, G. V., Dubey, J. P., and Su, C. (2008). Genotyping studies of Toxoplasma gondii isolates from Africa revealed that the archetypal clonal lineages predominate as in North America and Europe. Veterinary Parasitology, 155(3–4), 314–318. https://doi.org/10.1016/j.vetpar.2008.04.021

Zhu, J., et al. (2010). Molecular and serological diagnosis of Toxoplasma gondii infection in experimentally infected chickens. Veterinary Parasitology, 173(3–4), 197–201. https://doi.org/10.1016/j.vetpar.2010.07.006

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