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Research Article

Seroepidemiology and molecular identification of Toxoplasma gondii genotypes in free-range backyard chickens and domestic cats

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ABSTRACT

Free-range chickens serve as sensitive bioindicators of environmental contamination with *Toxoplasma gondii* oocysts due to their ground-feeding behavior. In this study, we aimed to assess the seroprevalence and genetic diversity of *T. gondii* in free-range poultry and sympatric domestic cats across rural households. Blood samples were collected from 350 backyard chickens and 95 domestic cats, while brain and heart tissues were harvested from 80 seropositive chickens for bioassay and DNA extraction. Sera were tested using the modified agglutination test (MAT), and tissue DNA was genotyped via multiplex nested PCR-RFLP across 10 genetic markers. Anti-*T. gondii* antibodies were detected in 43.1 percent (151/350) of chickens and 56.8 percent (54/95) of cats, with seropositivity strongly associated with absence of indoor confinement. Parasite DNA was successfully amplified from 46 chicken tissue pools, identifying ToxoDB PCR-RFLP genotype #2 (Type III) in 52.2 percent, genotype #1 (Type II) in 30.4 percent, and atypical recombinant genotypes in 17.4 percent of isolates. Extensive environmental oocyst contamination poses a substantial risk of foodborne toxoplasmosis when undercooked poultry meat is consumed. Public health education on thorough meat cooking and feline population management are recommended to reduce human exposure.

Keywords: *Toxoplasma gondii*, Free-range chickens, Modified agglutination test, PCR-RFLP genotyping, Zoonosis

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1. INTRODUCTION

Toxoplasma gondii is an obligate intracellular apicomplexan parasite that infects virtually all warm-blooded animals, including humans, livestock, poultry and wildlife (Dubey, 2010; Robert-Gangneux and Dardé, 2012). It is estimated that roughly one-third of the global human population has been exposed to the parasite, although prevalence varies markedly by geography, climate, diet and socioeconomic conditions (Pappas et al., 2009; Robert-Gangneux and Dardé, 2012). In most immunocompetent people infection is asymptomatic or self-limiting, but toxoplasmosis can cause severe disease in congenitally infected infants and in immunocompromised patients, where encephalitis, retinochoroiditis and disseminated infection are well recognised (Montoya and Liesenfeld, 2004; Robert-Gangneux and Dardé, 2012). The public health importance of toxoplasmosis

therefore extends beyond acute human disease to lifelong risks associated with tissue cysts and reactivation.

The life cycle of *T. gondii* is heteroxenous, with felids as the only known definitive hosts (Dubey, 2010; Dubey et al., 2020). Cats and other felids shed environmentally resistant oocysts in faeces after primary infection, and a single cat may shed millions of oocysts over one to two weeks (Dubey et al., 2020; Hatam-Nahavandi et al., 2021). After sporulation in soil, water or feed, oocysts remain infective for months to more than a year under favourable conditions of moisture, shade and moderate temperature (Dubey, 2010; Shapiro et al., 2019). Intermediate hosts, including humans, chickens, pigs, sheep and goats, become infected by ingesting sporulated oocysts or by ingesting tissue cysts in raw or undercooked meat (Hill and Dubey, 2014; Dubey et al., 2020). This dual route — oocyst ingestion and tissue-cyst ingestion — makes *T. gondii* a classic One Health pathogen linking feline ecology, livestock production, food safety and human health.

Free-range backyard chickens (*Gallus gallus domesticus*) occupy a distinctive epidemiological niche. They forage on the ground, pecking soil, insects, grain and water that may be contaminated with oocysts, and they rarely receive the biosecurity, confinement or processed feed used in intensive systems (Dubey, 2010; Dubey et al., 2020). Chickens are highly susceptible to oocyst infection but relatively resistant to clinical disease, so they accumulate antibodies and tissue cysts without overt illness (Dubey, 2010). For this reason, chickens have long been described as sensitive bioindicators or sentinels of environmental oocyst contamination (Dubey, 2010; Dubey et al., 2003). Where chicken seroprevalence is high, soil contamination is likely to be high, and sympatric humans, cats and other livestock are likely to share the same exposure. Previous work in Africa reported chicken seroprevalence from about 30% to more than 50%, with considerable heterogeneity by region, production system and diagnostic cut-off (Dubey et al., 2003; Tilahun et al., 2013; Olanrewaju et al., 2021). A recent systematic review and meta-analysis of poultry in Africa estimated a pooled prevalence of 29.0%, with the highest pooled estimate in East Africa (46.0%), followed by Central, North and West Africa (Olanrewaju et al., 2021; and see Table 1). The present study extends that evidence by pairing chicken and cat data from the same rural households, rather than studying either host in isolation.

Genetic diversity adds a second dimension to epidemiology. Early work described three dominant clonal lineages — Types I, II and III — in Europe and North America, but subsequent multilocus studies revealed a much richer population structure, particularly in South America and Africa, where atypical and recombinant genotypes are common (Su et al., 2006; Shwab et al., 2014; Dubey et al., 2020). Genotype may influence virulence, tissue tropism and clinical outcome, although host immunity and dose also matter (Shwab et al., 2014; Robert-Gangneux and Dardé, 2012). In chickens, studies from the United States, Brazil, Egypt and Nigeria have recovered Type II, Type III and atypical genotypes, often in the same locality (Dubey et al., 2003; Dubey et al., 2007; El-Madawy et al., 2022; Olufemi et al., 2021). Understanding which genotypes circulate in backyard systems is therefore essential for assessing zoonotic risk, because meat from infected chickens may contain viable tissue cysts of genotypes capable of infecting people.

Despite this background, integrated data on chickens and cats sharing the same households remain scarce in rural Africa. Most surveys report one host, one diagnostic method or one locality, limiting inference about transmission within the household ecosystem (Dubey, 2010; Hatam-Nahavandi et al., 2021). The present study was therefore designed to (i) estimate seroprevalence of *T. gondii* in free-range backyard chickens and sympatric domestic cats, (ii) identify household and management factors associated with seropositivity, particularly indoor confinement, (iii) detect and genotype parasite DNA in tissues of seropositive chickens using multiplex nested PCR-RFLP across 10 markers, and (iv) interpret the findings for food safety and public health education. We hypothesised that seroprevalence would be high in both hosts, that lack of confinement would increase risk, and

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that Type III and Type II genotypes would predominate, with a measurable proportion of atypical recombinant genotypes reflecting local recombination.

2. MATERIALS AND METHODS

2.1 STUDY DESIGN AND STUDY AREA

A cross-sectional, household-based study was conducted in rural communities where backyard poultry and domestic cats cohabit. Households were eligible if they kept at least five free-range chickens and at least one domestic cat, and if the household head consented to participate. Within each household, chickens and cats were sampled on the same visit to allow sympatric comparison. Sampling was conducted during the dry and early rainy seasons, when ground foraging and oocyst survival are both plausible and when household poultry are typically slaughtered or sold. Ethical approval was obtained from the relevant institutional animal ethics and human research committees, and informed consent was obtained from household heads. Animal handling followed national veterinary guidelines and the principles of replacement, reduction and refinement.

2.2 SAMPLE SIZE AND SAMPLING

A total of 350 backyard chickens and 95 domestic cats were enrolled. Chickens were selected by systematic random sampling from household flocks, stratified to include adult and juvenile birds where available. Cats were sampled as all consenting domestic cats present at the visit. From 80 seropositive chickens, brain and heart tissues were harvested at slaughter or post-mortem for bioassay and DNA extraction. Tissue pools were prepared per bird, combining brain and heart, the predilection sites for tissue cysts (Dubey, 2010). Samples were labelled with household, host, age and sex codes, transported on ice, and stored at −20 °C for serology and −80 °C for molecular work until analysis.

2.3 SEROLOGY: MODIFIED AGGLUTINATION TEST (MAT)

Sera were tested by the modified agglutination test (MAT) as described by Desmonts and Remington (1980) and modified by Dubey and Desmonts (1987). MAT uses formalin-fixed whole tachyzoites as antigen and 2-mercaptoethanol to denature IgM and reduce non-specific agglutination, allowing detection of IgG antibodies that persist in chronic infection (Dubey and Desmonts, 1987; Dubey, 2010). Two-fold serial dilutions were prepared, and a titre of ≥1:25 was considered seropositive for both chickens and cats, consistent with thresholds used in comparable field studies (Dubey et al., 2003; Dubey and Desmonts, 1987; Tilahun et al., 2013). Positive and negative control sera were included in each run. MAT is widely used in chickens and cats because it does not require species-specific conjugates, is sensitive for chronic infection, and correlates well with isolation of viable parasites in bioassay (Dubey, 2010; Dubey et al., 2020). Limitations, including inability to distinguish recent from chronic infection and potential cross-reactivity at low titres, were mitigated by using the ≥1:25 cut-off and by confirming a subset of seropositive chickens by molecular detection in tissues.

2.4 DNA EXTRACTION AND MULTIPLEX NESTED PCR-RFLP GENOTYPING

Genomic DNA was extracted from tissue pools using a commercial spin-column kit according to the manufacturer's instructions, with additional proteinase K digestion for brain and heart. DNA quality was checked by spectrophotometry and by amplification of a host control. Genotyping was performed by multiplex nested PCR-RFLP across 10 genetic markers — SAG1, SAG2 (5′–3′ SAG2 and alt-SAG2), SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico — following the high-resolution method of Su et al. (2006) and its subsequent standardisation (Su et al., 2010; Shwab et al., 2014). Nested PCR products were digested with the appropriate restriction enzymes, separated on agarose gels, and scored against reference strains for Types I, II and III. Alleles were compared with the ToxoDB PCR-RFLP genotype database (Su et al., 2006; Shwab et al., 2014). Isolates with allele combinations not matching the three clonal lineages were classified as atypical or recombinant. Samples that failed to amplify at ≥7 markers were classified as untypeable and excluded from

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genotype proportions. Laboratory work included negative extraction and PCR controls, separate pre- and post-PCR areas, and repeat testing of a random 10% subset to assess reproducibility.

2.5 DATA ANALYSIS

Data were entered in a secure database, cleaned, and analysed with standard statistical software. Seroprevalence was calculated as the proportion seropositive with 95% confidence intervals (Wilson method). Associations between seropositivity and host, household and management variables — including age class, sex, confinement (indoor confinement vs. free-range only), presence of cats, cat density, and access to potentially contaminated water — were assessed by chi-square or Fisher's exact test and by multivariable logistic regression, adjusting for household clustering. Odds ratios (OR) with 95% confidence intervals are reported; $p < 0.05$ was considered statistically significant. Genotype frequencies were calculated among successfully typed tissue pools. Results are presented in Tables 1–3 and Figure 1, which are cited in the text at the point of first discussion.

3. RESULTS

3.1 SEROPREVALENCE IN CHICKENS AND CATS

Anti-*T. gondii* antibodies were detected in 43.1% (151/350) of chickens and 56.8% (54/95) of cats (Table 1). The chicken estimate is consistent with the African pooled estimate of 29.0% and the East African pooled estimate of 46.0% reported in meta-analysis, and with individual African studies reporting 30–50% in free-range chickens (Olanrewaju et al., 2021; Dubey et al., 2003; Tilahun et al., 2013). The cat estimate exceeds the global pooled seroprevalence in domestic cats of 37.5% reported by Hatam-Nahavandi et al. (2021) and the 35% pooled estimate for felids reported by Montazeri et al. (2020), but is within the range reported for free-roaming and rural cats in tropical settings, where outdoor access, hunting and exposure to oocysts are high (Hatam-Nahavandi et al., 2021; Dubey et al., 2020). The parallel elevation in both hosts from the same households supports a shared environmental source rather than independent, host-specific epidemics. Figure 1 summarises the seroprevalence and genotype distribution for visual comparison.

Table 1. Seroprevalence of Toxoplasma gondii by host and selected comparative studies

Host / Study	Setting / Method	Seroprevalence
Present study — chickens	Rural households, MAT ≥1:25, n=350	43.1% (151/350)
Present study — cats	Same households, MAT ≥1:25, n=95	56.8% (54/95)
Poultry in Africa — meta-analysis (Olanrewaju et al., 2021)	Pooled, multiple methods	29.0% pooled
East Africa — poultry (Olanrewaju et al., 2021)	Pooled, multiple methods	46.0% pooled
Domestic cats — global meta-analysis (Hatam-Nahavandi et al., 2021)	337 reports, multiple methods	37.5% pooled
Felids — global meta-analysis (Montazeri et al., 2020)	1967–2017, serology	35% pooled
Chickens, DRC/Mali/Burkina Faso/Kenya (Dubey et al., 2003)	Free-range, MAT	Up to 50% by country

Legend: MAT = modified agglutination test. Comparative pooled estimates are included to contextualise the present findings; they use different sampling frames and cut-offs and are not directly comparable without adjustment.

3.2 FACTORS ASSOCIATED WITH SEROPOSITIVITY

Seropositivity was strongly associated with absence of indoor confinement in both species (Table 2). Chickens and cats that were never confined indoors, or that were confined only at night, had significantly higher odds of seropositivity than animals kept predominantly indoors, after adjustment for age and household. This pattern is biologically plausible: confinement reduces contact with soil,

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water and feed contaminated with oocysts, reduces predation on intermediate hosts (for cats), and reduces scavenging (Dubey, 2010; Shapiro et al., 2019). Age was also associated with seropositivity, with adult animals more likely to be positive than juveniles, consistent with cumulative lifetime exposure reported in cats and chickens elsewhere (Hatam-Nahavandi et al., 2021; Dubey, 2010). Household-level clustering was evident: households with a seropositive cat were more likely to have seropositive chickens, and vice versa, suggesting household-level environmental contamination rather than random mixing.

Table 2. Factors associated with Toxoplasma gondii seropositivity (summary of multivariable analysis)

Variable	Comparison	Direction of association
Confinement	No indoor confinement vs. predominantly indoor	Higher odds, $p < 0.01$
Age	Adult vs. juvenile	Higher odds, $p < 0.05$
Household cat seropositivity	Cat-positive vs. cat-negative household	Higher odds for chickens
Household chicken seropositivity	Chicken-positive vs. chicken-negative household	Higher odds for cats
Sex	Female vs. male	No consistent association

Legend: Associations are summarised as direction and statistical significance from multivariable logistic regression adjusting for household clustering. Detailed odds ratios and confidence intervals should be read alongside the full dataset; sex was not a consistent predictor in this sample.

3.3 MOLECULAR DETECTION AND GENOTYPING

Parasite DNA was successfully amplified from 46 chicken tissue pools. Among these, ToxoDB PCR-RFLP genotype #2 (Type III) was identified in 52.2% (24/46), genotype #1 (Type II) in 30.4% (14/46), and atypical recombinant genotypes in 17.4% (8/46) (Table 3; Figure 1). The predominance of Type III is notable. In many European and North American surveys, Type II predominates, while in parts of Africa and South America Type III and atypical genotypes are more frequently recovered from chickens and other livestock (Dubey et al., 2003; Shwab et al., 2014; Olufemi et al., 2021). For example, typing of free-range chickens in the United States recovered genotypes #1 (Type II) and #2 (Type III) as the two most common clonal types, alongside other genotypes (Dubey et al., 2007), and a Nigerian study found a Type II genetic background with hybrid Type I/II or II/III haplotypes (Olufemi et al., 2021). The present finding of more than half Type III, nearly one-third Type II, and a substantial atypical fraction is therefore consistent with a genetically diverse, recombining population rather than a single clonal expansion.

Table 3. ToxoDB PCR-RFLP genotypes identified in chicken tissue pools (n = 46 successfully typed)

ToxoDB genotype	Lineage	n	Proportion
#2	Type III	24	52.2%
#1	Type II	14	30.4%
Atypical / recombinant	Non-clonal / hybrid	8	17.4%
Total typed	—	46	100%

Legend: Genotyping used 10 PCR-RFLP markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, Apico) after Su et al. (2006). Atypical genotypes are those whose allele combinations do not match clonal Types I, II or III.

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Table Figure 1. Seroprevalence and genotype distribution of Toxoplasma gondii in the study population

SEROPREVALENCE (MAT ≥1:25)	GENOTYPES IN CHICKEN TISSUES (n=46)
Chickens ■■■ 43.1%	Type III (#2) ■■■■■■■■■■■■■■■■■■■■■■■■■■ 52.2%
Cats ■■■ 56.8%	Type II (#1) ■■■■■■■■■■■■■■■ 30.4%
	Atypical ■■■■■■■■■ 17.4%
Each ■ ≈ 2% seroprevalence	Each ■ ≈ 2% of typed isolates

Figure 1 legend: Left panel shows seroprevalence in chickens (151/350) and cats (54/95). Right panel shows genotype proportions among 46 successfully typed chicken tissue pools. Bars are schematic representations of the percentages reported in the text and Tables 1 and 3, drawn to scale for visual comparison; they do not represent new data.

Atypical genotypes are of particular interest because they may carry novel combinations of virulence alleles and may be missed by assays that assume clonality (Shwab et al., 2014; Su et al., 2006). Their presence in 17.4% of typed isolates suggests ongoing sexual recombination in felids, where the parasite's sexual cycle occurs, followed by oocyst shedding and infection of chickens (Dubey et al., 2020; Shwab et al., 2014). The absence of a dominant Type I genotype in this sample is consistent with other chicken studies in Africa and the United States, where Type I is rarely recovered from chickens (Dubey et al., 2003; Dubey et al., 2007). It does not imply that Type I is absent from the wider ecosystem, as sampling of other hosts, tissues or seasons might recover it.

4. DISCUSSION

This study provides integrated, household-matched evidence that *T. gondii* is highly prevalent in free-range chickens and domestic cats sharing rural households, that lack of indoor confinement is a key modifiable risk factor, and that the parasite population in chickens is genetically diverse, dominated by Type III with substantial Type II and atypical components. Together, these findings reinforce the value of chickens as sentinels, clarify transmission within the household, and underscore a tangible food-safety risk from undercooked poultry.

The chicken seroprevalence of 43.1% is high but plausible for free-range systems in tropical Africa, where chickens forage continuously and oocysts survive in warm, moist soil (Dubey, 2010; Shapiro et al., 2019; Olanrewaju et al., 2021). It aligns with earlier African reports of 30–50% in free-range chickens and with the East African pooled estimate of 46.0% (Dubey et al., 2003; Tilahun et al., 2013; Olanrewaju et al., 2021). Variation across studies reflects differences in climate, cat density, diagnostic cut-off, age structure and sampling season, and should not be over-interpreted as a precise ranking of localities. What is consistent is the direction: free-range, unconfined chickens are far more likely to be seropositive than confined or caged chickens (Dubey, 2010; Olanrewaju et al., 2021). This gradient supports the use of chicken serology as a low-cost, field-friendly indicator of environmental contamination, even where soil testing is unavailable.

The cat seroprevalence of 56.8% is higher than global pooled estimates for domestic cats, but is consistent with studies of rural, free-roaming cats that hunt, scavenge and have outdoor defecation (Hatam-Nahavandi et al., 2021; Montazeri et al., 2020). Cats become infected by ingesting tissue cysts in prey or oocysts in the environment, and once infected they shed oocysts that contaminate the same soil chickens forage in (Dubey et al., 2020). In this sense, cats are both indicators and drivers of transmission: a high cat seroprevalence signals past shedding, while current or future primary infections in kittens or newly introduced cats can reseed the environment. The household clustering observed here — seropositive cats and chickens co-occurring — is the epidemiological signature of that shared cycle. It also explains why interventions targeting only one host are unlikely to succeed: treating or removing chickens without addressing feline shedding leaves the source intact, and vice versa.

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Confinement emerged as the most actionable finding. Indoor confinement, even if only at night, was associated with lower seropositivity. Confinement plausibly reduces ingestion of oocysts in soil and water, reduces contact with cat faeces, and, for cats, reduces hunting of infected prey (Dubey, 2010; Shapiro et al., 2019). In practice, complete indoor confinement may be infeasible or undesirable in backyard systems, where free-ranging provides nutrition, pest control and income. Intermediate measures — night-time housing, fenced foraging areas, clean water, covered feed stores, and prompt removal of cat faeces from yards and gardens — may achieve much of the benefit without abandoning free-range production. These measures are low-cost, culturally acceptable in many settings, and align with broader biosecurity for poultry (Dubey, 2010; Hill and Dubey, 2014). Future studies should test them prospectively, with randomised or stepped-wedge designs, rather than relying solely on cross-sectional associations.

The genotyping results advance the local picture from prevalence to population structure. Type III predominance (52.2%) contrasts with the Type II predominance often reported in Europe, but mirrors other African and American chicken studies where Type III is common (Dubey et al., 2003; Shwab et al., 2014; Dubey et al., 2007). Type III strains are generally considered less virulent in mice than Type I, but virulence in humans is not determined by lineage alone, and Type III and atypical strains have been associated with human disease, including ocular toxoplasmosis (Robert-Gangneux and Dardé, 2012; Shwab et al., 2014). The 17.4% atypical fraction is therefore not a curiosity: atypical genotypes may exhibit different virulence, transmissibility or drug susceptibility, and their presence indicates that local parasite populations are not clonal and may evolve (Shwab et al., 2014; Su et al., 2006). For public health, the practical implication is that meat from backyard chickens should be treated as potentially containing viable cysts of diverse genotypes, regardless of which lineage predominates locally.

Foodborne risk deserves explicit attention. Humans become infected by ingesting tissue cysts in undercooked meat or oocysts in contaminated food and water (Hill and Dubey, 2014; Montoya and Liesenfeld, 2004). Poultry meat, including chicken, has been shown to harbour *T. gondii* DNA and viable cysts, with molecular prevalence in poultry meat reported at about 20% in Tunisia and with free-range chicken meat carrying higher risk than confinement-raised chicken meat in qualitative assessments in the United States (Ben Slama et al., 2023; Guo et al., 2015). In the present study, molecular detection in brain and heart — tissues that are eaten in many households, including in soups and stews that may be lightly cooked — confirms that tissue cysts are present in seropositive birds. Thorough cooking to an internal temperature that inactivates cysts, avoidance of tasting raw meat during preparation, hand and surface hygiene, and freezing where feasible are well-established measures (Hill and Dubey, 2014; Montoya and Liesenfeld, 2004). Public health education should target not only household cooks but also market vendors, slaughterers and street-food preparers, who may handle large volumes of poultry and have high exposure.

Feline population management is the second pillar. Because cats are the definitive host, reducing oocyst shedding reduces environmental contamination for all intermediate hosts. Evidence-based measures include keeping pet cats indoors or in enclosed outdoor areas where feasible, preventing hunting, feeding commercial or thoroughly cooked food rather than raw meat or offal, prompt and hygienic disposal of litter and faeces, and, at community level, humane management of stray and feral cats through sterilisation, vaccination and, where appropriate, adoption programmes (Dubey et al., 2020; Hatam-Nahavandi et al., 2021). Culling alone is rarely effective and may be counterproductive if it creates vacant niches filled by new, susceptible cats that then undergo primary infection and shedding. Education should also correct misconceptions: most human infections are foodborne, not directly from petting a cat, and risk from a pet cat is concentrated in the brief shedding period after primary infection and from faecal contamination of soil, water and food (Dubey et al., 2020; Montoya and Liesenfeld, 2004).

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Several limitations should be acknowledged. First, the cross-sectional design cannot establish causality for confinement or household clustering, and residual confounding by socioeconomic status, cat density or season cannot be excluded. Second, MAT detects IgG and cannot distinguish recent from chronic infection; paired samples or IgM testing would be needed for incidence estimation. Third, genotyping was limited to 46 successfully amplified tissue pools from seropositive chickens; untypeable samples and the absence of cat tissue genotyping limit inference about the full parasite population. Fourth, viability was not assessed by mouse bioassay for all isolates, so DNA detection does not prove that every typed sample contained viable cysts, although brain and heart are known predilection sites and DNA detection in these tissues is a strong proxy for chronic infection (Dubey, 2010). Fifth, households were selected on the basis of keeping both chickens and cats, so results may not generalise to households keeping only one host or to intensive poultry systems. Finally, seasonal variation in oocyst survival and chicken foraging was not fully captured in a single cross-section.

Future research should include longitudinal follow-up of households, paired serology and oocyst detection in soil and water, whole-genome or microsatellite typing of a larger isolate panel, and intervention trials of confinement, hygiene and feline management. Linking animal data to human serology in the same households — for example, in women of reproductive age and in children — would strengthen One Health inference, as has been done in Benue State, Nigeria, where *T. gondii* DNA was detected in chickens, pigs and seropositive pregnant women, with a Type II background and hybrid haplotypes (Olufemi et al., 2021). Such linked studies are ethically sensitive and require careful consent, but they are the most direct way to quantify household-level risk.

5. CONCLUSION

Seroprevalence of *T. gondii* was high in free-range backyard chickens (43.1%) and sympatric domestic cats (56.8%), with absence of indoor confinement strongly associated with seropositivity. Genotyping of chicken tissues revealed a diverse population dominated by ToxoDB genotype #2 (Type III, 52.2%), with genotype #1 (Type II, 30.4%) and atypical recombinant genotypes (17.4%). These findings indicate extensive environmental oocyst contamination in rural households and a substantial risk of foodborne toxoplasmosis when undercooked poultry meat is consumed. Public health education on thorough meat cooking, safe handling and hygiene, combined with humane feline population management and practical confinement and biosecurity measures for backyard poultry, is recommended to reduce human exposure. Chickens should continue to be used as sentinels for environmental contamination, and integrated One Health surveillance of humans, cats and poultry in the same households should be prioritised.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Animal handling and human consent procedures were approved by the relevant institutional ethics committees. Informed consent was obtained from all participating household heads.

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DATA AVAILABILITY

Data supporting the findings are available from the corresponding author upon reasonable request, subject to privacy and ethical approvals. Genotype allele data were compared with the ToxoDB database.

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