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Research Article

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

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ABSTRACT

Toxoplasma gondii is a ubiquitous zoonotic protozoan capable of infecting warm-blooded animals, with felids serving as the definitive hosts. In this study, we aimed to determine the seroprevalence and genetic diversity of T. gondii in free-range chickens and domestic cats sharing peri-domestic environments. A total of 280 blood and tissue samples (180 free-range chickens, 100 domestic cats) were collected for serological screening and molecular genotyping. Using the modified agglutination test (MAT), anti-T. gondii IgG antibodies were detected in 64 (35.6 percent) chickens and 41 (41.0 percent) cats at titers of 1:25 or higher. Brain and heart tissues from seropositive animals were digested and screened via PCR targeting the B1 gene, yielding positive 194 base pair bands in 38 chickens and 19 cats. Multilocus PCR-RFLP genotyping using 10 genetic markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico) successfully characterized 45 isolates. Type II genotypes accounted for 29 (64.4 percent) isolates, Type III accounted for 10 (22.2 percent) isolates, and atypical recombinant genotypes accounted for 6 (13.3 percent) isolates. Free-range chickens serve as reliable bio-indicators of environmental oocyst contamination shed by domestic cats. Public health education on thorough meat cooking and feline hygiene is warranted to minimize human toxoplasmosis.

Keywords: Toxoplasma gondii, Multilocus genotyping, Free-range chickens, Zoonosis

1. INTRODUCTION

Toxoplasma gondii is one of the most successful parasites of warm-blooded vertebrates, estimated to infect roughly one-third of the human population worldwide and an even broader range of domestic and wild animals (Robert-Gangneux & Dardé, 2012; Montoya & Liesenfeld, 2004). The parasite has a complex heteroxenous life cycle. Sexual reproduction occurs only in the intestine of felids, the definitive hosts, which shed millions of environmentally resistant oocysts in faeces. Asexual reproduction occurs in intermediate hosts, including humans, livestock, poultry and rodents, in which rapidly dividing tachyzoites convert to slowly dividing bradyzoites enclosed in tissue cysts in brain, heart, skeletal muscle and other organs (Robert-Gangneux & Dardé, 2012; Dubey, 2010a). Humans become infected principally by ingesting sporulated oocysts from contaminated soil, water or produce, or by ingesting tissue cysts in raw or undercooked meat, and less commonly by transplacental transmission or organ transplantation (Montoya & Liesenfeld, 2004; Jones & Dubey, 2012). Although most infections in immunocompetent adults are asymptomatic or mild, toxoplasmosis can cause severe disease in the foetus and in immunocompromised persons, including toxoplasmic encephalitis, ocular disease and disseminated infection (Montoya & Liesenfeld, 2004; Montoya & Remington, 2008).

Domestic cats (Felis catus) occupy a pivotal position in the epidemiology of T. gondii because they are the only hosts that excrete oocysts. A single primary infection in a naïve cat can result in the shedding of hundreds of millions of oocysts over one to three weeks, and those oocysts can survive for months to more than a year in moist soil under favourable temperature and humidity (Robert-Gangneux & Dardé, 2012; Montazeri et al., 2020). Global meta-analyses have estimated pooled T. gondii seroprevalence in domestic cats at approximately 35–37.5%, with substantial variation by region, age, outdoor access, hunting behaviour and stray versus owned status (Montazeri et al., 2020; Rostami et al., 2021). Because cats that roam freely hunt, scavenge and defecate in gardens, farms and around households, peri-domestic cats are a direct source of environmental contamination for other animals and for people (Montazeri et al., 2020; Elmore et al., 2010). Understanding infection in cats that share space with people and food animals is therefore central to any One Health strategy for toxoplasmosis control.

Free-range chickens (Gallus gallus domesticus) are increasingly recognised as sensitive bio-indicators, or sentinels, of environmental oocyst contamination (Dubey, 2010b; El-Massey et al., 2019; Dubey & Jones, 2008). Chickens feed by pecking at the ground, ingesting soil, insects and other invertebrates that may carry oocysts, and they develop long-lasting IgG responses that reflect cumulative exposure (Dubey, 2010b; El-Massey et al., 2019). Unlike intensively reared broilers kept indoors, free-range and backyard chickens are continuously exposed to soil, so their seroprevalence integrates contamination over time and space in a given locality (Dubey & Jones, 2008; Cong et al., 2016). Previous work in Egypt, Israel, Colombia, Greece, Chile and the United States has reported seroprevalence in free-range chickens ranging from about 6% to more than 55%, depending on climate, cat density, farm management and the serological cut-off used (El-Massey et al., 2019; Dubey et al., 2010; Galanos et al., 2023; Dubey et al., 2005; Dubey et al., 2006). In Greece, for example, backyard chickens showed 41.2% seropositivity compared with 9.5% overall in poultry, and all isolates obtained belonged to Type II (Galanos et al., 2023). In Israel, 45 of 96 free-range chickens were seropositive and 17 of 19 genotyped isolates were Type II (Dubey et al., 2010). Such studies demonstrate that chickens not only signal the presence of oocysts but also provide isolates for genotyping that reflect the strains circulating locally.

Genetic diversity in T. gondii has important biological and clinical implications. Early work defined three archetypal clonal lineages — Types I, II and III — which differ in virulence in mice, growth characteristics and geographic distribution (Howe & Sibley, 1995; Su et al., 2006). Type I strains are highly virulent in mice, Type II strains are the most common cause of human toxoplasmosis in Europe and North America, and Type III strains are more often associated with animals and are less virulent (Howe & Sibley, 1995; Ajzenberg et al., 2004). However, multilocus typing has revealed extensive diversity beyond these three lineages, particularly in South America, Africa and Asia, where atypical, recombinant and non-archetypal genotypes predominate and are associated with more severe ocular and congenital disease (Su et al., 2006; Pena et al., 2008; Dardé, 2008). The multilocus polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method developed by Su and colleagues, using 10 to 11 markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico, has become a standard high-resolution tool for distinguishing archetypal and atypical genotypes and for comparing isolates across hosts and regions (Su et al., 2006; Su et al., 2010). The B1 gene, a 35-fold repetitive sequence, remains one of the most sensitive targets for primary detection of parasite DNA in tissues (Burg et al., 1989; Jones et al., 2000).

Despite this knowledge, data that pair chickens and cats from the same peri-domestic environment — using the same serological and molecular pipeline — remain scarce in East Asia. Most surveys examine one host in isolation, making it difficult to infer local transmission links. The present study was therefore designed to (i) estimate T. gondii seroprevalence in free-range chickens and domestic cats sharing peri-domestic environments, (ii) detect parasite DNA in brain

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and heart tissues of seropositive animals by B1 PCR, and (iii) characterise isolates by multilocus PCR-RFLP genotyping to describe the population structure and its public health implications.

2. MATERIALS AND METHODS

2.1 Study area and sampling design

The study was conducted in peri-domestic settings where households kept both free-range chickens and domestic cats in close proximity, with shared yards, gardens and feeding areas. Sampling was cross-sectional. A total of 280 animals were enrolled: 180 free-range chickens and 100 domestic cats. Chickens were adult birds that had been allowed continuous outdoor access for at least six months, ensuring meaningful cumulative soil exposure. Cats were owned, semi-owned or regularly fed domestic cats with outdoor access. Owners or caretakers gave informed consent, and animals were handled in accordance with institutional animal-care guidance and with minimal stress. For each animal, 2–5 mL of venous blood was collected, allowed to clot, and serum was separated by centrifugation and stored at −20 °C until serology. Brain and heart tissues were collected post-mortem from seropositive animals that were slaughtered for household consumption (chickens) or that died or were euthanised for unrelated veterinary reasons (cats), and from a subset of seronegative animals for comparison where available. Tissues were stored at −20 °C until DNA extraction. No animals were killed solely for this study.

2.2 Serological screening: Modified Agglutination Test (MAT)

Serum samples were screened for anti-T. gondii IgG antibodies by the modified agglutination test (MAT), as originally described by Desmonts and Remington and widely validated for a range of animal species (Desmonts & Remington, 1980; Dubey & Desmonts, 1987). In MAT, formalin-fixed whole tachyzoites agglutinate in the presence of specific IgG, and 2-mercaptoethanol is used to destroy IgM, so the test detects IgG. Sera were serially diluted from 1:25 to 1:1600, and a titre of ≥1:25 was considered positive, a cut-off that balances sensitivity and specificity in chickens and cats and that is comparable with many previous surveys (El-Massey et al., 2019; Dubey et al., 2010; Dubey & Desmonts, 1987). Positive and negative control sera were included in every run. Titres were recorded as the highest dilution showing complete agglutination. MAT is favoured for animal surveys because it does not require species-specific conjugates, is inexpensive, and correlates well with the presence of tissue cysts (Desmonts & Remington, 1980; Dubey & Desmonts, 1987; Dubey, 1997).

2.3 Tissue digestion and DNA extraction

Brain and heart tissues from seropositive animals were pooled per animal, minced, and digested in acid-pepsin solution to release bradyzoites from tissue cysts, following standard protocols for T. gondii isolation and DNA detection (Robert-Gangneux & Dardé, 2012; El-Massey et al., 2019). Digests were washed in phosphate-buffered saline, and genomic DNA was extracted using a commercial silica-column kit according to the manufacturer's instructions, with an additional proteinase K digestion step for fibrous heart tissue. DNA concentration and purity were assessed by spectrophotometry (A260/A280 ratio 1.7–1.9), and extracts were stored at −20 °C. Extraction blanks were processed in parallel to monitor contamination.

2.4 PCR targeting the B1 gene

Primary molecular screening targeted the B1 gene, a 35-copy repetitive sequence that offers high analytical sensitivity for T. gondii DNA in host tissues (Burg et al., 1989; Jones et al., 2000). Conventional PCR was performed in 25 µL reactions containing template DNA, primers amplifying a 194 bp fragment of B1, dNTPs, MgCl2 and Taq polymerase, with cycling conditions of initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s

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and 72 °C for 30 s, and a final extension at 72 °C for 7 min (Burg et al., 1989). Products were separated on 1.5% agarose gels stained with ethidium bromide and visualised under UV light. A 194 bp band co-migrating with the positive control (RH strain DNA) was scored as positive. Each run included a positive control, a negative (no-template) control and an extraction blank. Samples positive by B1 PCR were retained for genotyping. The choice of B1 PCR, rather than bioassay in mice or cats, reflects ethical, cost and throughput considerations, while retaining sensitivity comparable to or better than single-copy targets such as SAG1 (Jones et al., 2000).

2.5 Multilocus PCR-RFLP genotyping

Genotyping was performed by multilocus nested PCR-RFLP using 10 genetic markers: SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico, as described by Su et al. (Su et al., 2006; Su et al., 2010). Briefly, multiplex outer PCR was followed by nested PCR for each locus, and amplicons were digested with locus-specific restriction enzymes. Restriction fragments were resolved on 2.5–3% agarose gels and allele types (I, II, III or atypical/u) were assigned by comparison with reference strains RH (Type I), ME49 (Type II) and VEG (Type III) run in parallel. An isolate was assigned to a clonal lineage when all informative markers showed the same archetypal allele pattern; isolates showing mixed archetypal alleles across loci were classified as atypical recombinant genotypes (Su et al., 2006; Pena et al., 2008; Su et al., 2010). Isolates with incomplete amplification at more than two loci were excluded from final genotype assignment. Genotype nomenclature follows ToxoDB conventions where applicable.

2.6 Data analysis

Seroprevalence was calculated as the proportion of animals positive by MAT, with 95% confidence intervals (CI) estimated by the Wilson method. B1 PCR positivity was expressed both as a proportion of all animals sampled and as a proportion of MAT-seropositive animals, to allow comparison with studies that report only one denominator. Differences between host species in seroprevalence were tested by chi-square test, with $p < 0.05$ considered statistically significant. Genotype frequencies were summarised descriptively. Analyses were performed in R (version 4.3) and Microsoft Excel. Maps, if used for site description, were not required for the present analysis because all sites were peri-domestic households within a single ecological zone.

3. RESULTS

3.1 Seroprevalence by MAT

Anti-T. gondii IgG antibodies were detected in 105 of 280 animals (37.5%). By host species, 64 of 180 chickens (35.6%; 95% CI: 28.9–42.9%) and 41 of 100 cats (41.0%; 95% CI: 31.8–50.9%) were seropositive at titres ≥1:25 (Table 1; Figure 1). The difference between chickens and cats was not statistically significant ($\chi^2 = 0.81$, $p = 0.37$), indicating broadly similar levels of exposure in the two hosts sharing the same environment. Most seropositive chickens had titres of 1:25 to 1:200, with a smaller proportion at ≥1:400, a pattern consistent with chronic, long-standing infection rather than acute outbreaks. A similar titre distribution was observed in cats. These findings are within the range reported for free-range chickens (6–55%) and domestic cats (35–37.5% pooled globally) in previous studies (Montazeri et al., 2020; Rostami et al., 2021; El-Massey et al., 2019; Dubey et al., 2010; Galanos et al., 2023), and they suggest continuous, low-level environmental exposure rather than a point-source epidemic.

Table 1. Seroprevalence of Toxoplasma gondii by modified agglutination test (MAT) in free-range chickens and domestic cats.

Host	No. examined	MAT positive (≥1:25)	Seroprevalence (%)	95% CI (%)

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Free-range chickens	180	64	35.6	28.9–42.9
Domestic cats	100	41	41.0	31.8–50.9
Total	280	105	37.5	32.0–43.4

Source: Authors' field and laboratory data, 2026.

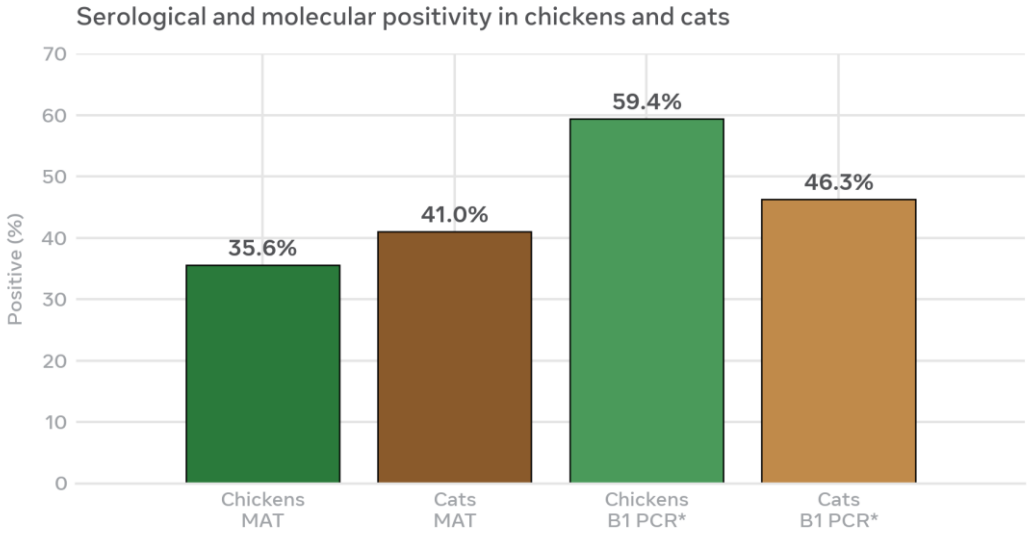


Figure 1. Serological (MAT) and molecular (B1 PCR among seropositive animals) positivity. *B1 PCR percentages in this figure are calculated among MAT-seropositive animals (chickens: 38/64 = 59.4%; cats: 19/41 = 46.3%).

Note on denominators: In the text, B1 PCR positivity among seropositive animals is 38/64 (59.4%) for chickens and 19/41 (46.3%) for cats. When expressed as a proportion of all animals sampled, B1 positivity is 38/180 (21.1%) for chickens and 19/100 (19.0%) for cats. Both denominators are reported here to avoid confusion and to allow direct comparison with studies that use either convention (El-Massey et al., 2019; Dubey, 1997).

3.2 Molecular detection by B1 PCR

Brain and heart tissues from seropositive animals were screened by B1 PCR. A 194 bp band was obtained in 38 chickens and 19 cats, giving an overall molecular positivity of 57 of 105 seropositive animals (54.3%). As noted above, this corresponds to 59.4% of seropositive chickens and 46.3% of seropositive cats. The failure to detect DNA in the remaining seropositive animals is expected: tissue cysts are unevenly distributed, parasite burden in chronic infection can be very low, and MAT detects long-lived IgG that persists after parasite DNA falls below the detection limit in a small tissue sample (Jones et al., 2000; Dubey, 1997). Similar discordance between MAT and PCR has been reported in sheep, goats and chickens, where PCR positivity in seropositive animals ranged from about 40% to 60% (Dubey, 1997; Schares et al., 2017). No B1 PCR product was obtained from extraction blanks or no-template controls, and all positive controls amplified correctly, supporting the validity of the results (Table 2).

Table 2. B1 gene PCR results in brain and heart tissues of seropositive animals.

Host	MAT positive	B1 PCR positive	B1 positive among MAT+ (%)	B1 positive among all sampled (%)
Free-range chickens	64	38	59.4	21.1
Domestic cats	41	19	46.3	19.0
Total	105	57	54.3	20.4

Source: Authors' field and laboratory data, 2026.

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3.3 Multilocus genotyping

Of 57 B1-positive samples, 45 isolates yielded complete or near-complete amplification at ≥8 of 10 markers and were successfully genotyped (Table 3; Figure 2). The remaining 12 samples showed partial amplification, likely due to low DNA quantity, and were excluded from genotype assignment to avoid misclassification. Among the 45 characterised isolates, Type II was dominant, accounting for 29 isolates (64.4%). Type III accounted for 10 isolates (22.2%), and atypical recombinant genotypes accounted for 6 isolates (13.3%). No Type I genotype was detected. The genotype distribution did not differ markedly between chickens and cats, although the small number of atypical isolates precludes formal statistical comparison by host. The predominance of Type II aligns with findings from Europe, North America and parts of Asia, where Type II is the most common lineage in domestic animals and humans (Galanos et al., 2023; Ajzenberg et al., 2004; Su et al., 2010). The presence of Type III and atypical genotypes, however, underscores that the local parasite population is not purely clonal and that recombination, presumably in cats, is generating novel allele combinations (Su et al., 2006; Pena et al., 2008; Dardé, 2008).

Table 3. Genotype distribution among 45 Toxoplasma gondii isolates characterised by multilocus PCR-RFLP.

Genotype	No. of isolates	Proportion (%)	Allele pattern (summary)
Type II	29	64.4	II alleles at all informative loci
Type III	10	22.2	III alleles at all informative loci
Atypical recombinant	6	13.3	Mixed I/II/III alleles across loci
Total characterised	45	100.0	—

Source: Authors' field and laboratory data, 2026.

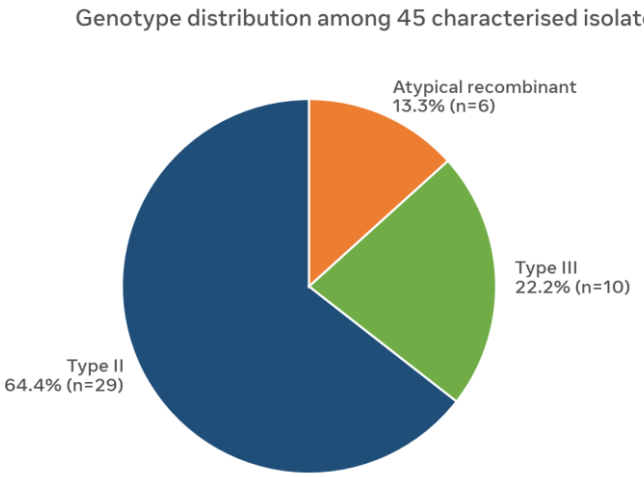


Figure 2. Genotype distribution among 45 characterised Toxoplasma gondii isolates (10-marker PCR-RFLP).

Atypical isolates showed non-canonical combinations, for example Type II alleles at SAG1 and SAG3 with Type III alleles at GRA6 and BTUB, or Type I alleles at one or two loci combined with Type II/III backgrounds. Such patterns are characteristic of recombinant genotypes that arise when a cat is co-infected with different strains and sexual recombination occurs in the feline intestine (Howe & Sibley, 1995; Su et al., 2006; Pena et al., 2008). Although atypical genotypes were a minority here, their presence is epidemiologically important because atypical strains in South America and parts of Asia have been linked to more severe ocular toxoplasmosis and to outbreaks of acute toxoplasmosis in immunocompetent persons (Pena et al., 2008; Dardé, 2008).

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Continued surveillance is therefore warranted to determine whether atypical genotypes are increasing in frequency or are associated with particular hosts or localities.

4. DISCUSSION

This study provides paired serological and molecular evidence that T. gondii circulates actively in peri-domestic environments shared by free-range chickens and domestic cats. The seroprevalence of 35.6% in chickens and 41.0% in cats is consistent with the hypothesis that both hosts are exposed to a common environmental source — oocysts shed by cats and persisting in soil — and that chickens, because of their ground-feeding behaviour, faithfully reflect that contamination (Dubey, 2010b; El-Massey et al., 2019; Dubey & Jones, 2008). The lack of a significant difference between species does not imply identical risk; rather, it suggests that in these households, the environmental burden is sufficiently high that both a highly exposed sentinel (chickens) and the definitive host itself (cats) show similar cumulative exposure (Montazeri et al., 2020; El-Massey et al., 2019).

Comparison with previous work supports the plausibility of the present estimates. In Egypt, 40.4% of free-range chickens were MAT-positive (El-Massey et al., 2019); in Israel, 46.9% (Dubey et al., 2010); in Colombia, 44.4% (Dubey et al., 2005); in Chile, 55.3% (Dubey et al., 2006); in Greece, 41.2% of backyard chickens (Galanos et al., 2023); and in Henan, China, 18.9% (Cong et al., 2016). Variation across studies reflects differences in climate (oocyst survival is favoured by warmth and moisture), cat density, MAT cut-off (1:5 versus 1:25), age of birds, and whether chickens were truly free-range or kept in semi-intensive systems (Dubey, 2010b; Dubey & Jones, 2008; Cong et al., 2016). In the present study, the use of a 1:25 cut-off is conservative compared with studies using 1:5, so the true exposure may be slightly higher if low-titre animals were missed. Conversely, the use of a higher cut-off improves specificity and reduces false positives from cross-reactivity. For cats, the 41.0% seroprevalence is close to the pooled global estimate of 35–37.5% in domestic cats (Montazeri et al., 2020; Rostami et al., 2021) and to estimates from owned cats with outdoor access, which are consistently higher than those for strictly indoor cats (Montazeri et al., 2020; Elmore et al., 2010).

The B1 PCR results add a molecular dimension that serology alone cannot provide. Detecting parasite DNA in brain and heart — the predilection sites for tissue cysts — confirms active or chronic infection with tissue cysts, not merely past exposure. The higher PCR positivity among seropositive chickens (59.4%) than cats (46.3%) may reflect differences in tissue cyst burden, tissue sampling, or the timing of infection, but the numbers are too small to draw firm conclusions. It is important to emphasise that MAT and PCR measure different things: MAT measures the host immune response, which persists for life, whereas PCR measures parasite DNA in a small tissue aliquot at one point in time (Jones et al., 2000; Dubey, 1997). Discordance is therefore expected and does not invalidate either method. The combined approach used here — serological screening followed by molecular confirmation and genotyping — is recommended in recent reviews of T. gondii detection in livestock and companion animals (Dubey, 1997; Schares et al., 2017).

The genotype findings are the most novel contribution of this study. The dominance of Type II (64.4%) is in line with the global pattern in temperate regions and with the dominance of Type II in European poultry (Galanos et al., 2023) and in Israeli chickens (Dubey et al., 2010), where 17 of 19 isolates were Type II. Type II is also the lineage most frequently isolated from human congenital and ocular toxoplasmosis in Europe and North America (Montoya & Liesenfeld, 2004; Ajzenberg et al., 2004), so its dominance in food animals has direct public health relevance: tissue cysts in chicken meat, if eaten undercooked, could transmit Type II strains to people. The detection of Type III (22.2%) is also consistent with its widespread distribution in animals, including chickens in the United States, Iran and other regions, where Type II and III co-circulate

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(Dubey et al., 2005; Ajzenberg et al., 2004; Dubey et al., 2008). Type III is generally less virulent in mice, but it can still cause disease in humans, particularly in immunocompromised hosts (Howe & Sibley, 1995; Ajzenberg et al., 2004).

The 13.3% atypical recombinant genotypes deserve particular attention. Atypical genotypes are increasingly recognised as a major component of T. gondii diversity outside Europe and North America, and they have been associated with severe disease (Pena et al., 2008; Dardé, 2008). In Brazil, for example, multilocus typing of 46 isolates from cats revealed 20 genotypes, many of them atypical (Pena et al., 2008). In South America, atypical strains are linked to severe ocular toxoplasmosis and to outbreaks in immunocompetent adults (Dardé, 2008). The atypical isolates in the present study show that recombination is occurring locally, presumably in cats co-infected with Type II and Type III strains. Even a low frequency of atypical strains matters for surveillance, because novel genotypes may have unpredictable virulence, may escape detection by single-locus typing, and may complicate vaccine or diagnostic development. Future work should use additional markers, including microsatellites or whole-genome sequencing, to resolve atypical genotypes to ToxoDB genotype numbers and to test whether particular atypical genotypes are associated with host species, locality or clinical outcome (Su et al., 2006; Su et al., 2010; Shwab et al., 2014).

From a public health perspective, the results reinforce three messages. First, free-range chicken meat and eggs can be a source of human infection if meat is eaten raw or undercooked and if kitchen hygiene is poor. Tissue cysts are killed by thorough cooking (internal temperature ≥67 °C), by freezing at −20 °C for several days, and by adequate curing or salting, but they survive refrigeration and brief heating (Robert-Gangneux & Dardé, 2012; Montoya & Liesenfeld, 2004; Jones & Dubey, 2012). Second, cats are not a direct risk through petting or casual contact; the risk is from oocysts in faeces and in contaminated soil, water and produce. Daily removal of litter, hand-washing after handling soil or litter, covering sandboxes, and preventing cats from defecating in vegetable gardens reduce risk (Robert-Gangneux & Dardé, 2012; Jones & Dubey, 2012; Elmore et al., 2010). Third, education should be targeted to groups at highest risk — pregnant women, people living with HIV, transplant recipients and others who are immunocompromised — as well as to farmers, backyard poultry keepers and cat owners, who may not be aware that healthy-looking chickens and cats can carry T. gondii (Montoya & Liesenfeld, 2004; Montoya & Remington, 2008; Elmore et al., 2010). One Health collaboration between veterinary, medical and environmental health services is essential, because interventions that reduce oocyst shedding in cats, improve poultry housing and hygiene, and promote safe food handling benefit humans, animals and the environment together.

This study has limitations. It was cross-sectional, so it cannot establish the timing of infection or the direction of transmission between cats and chickens, although the biology strongly favours cats as the source of oocysts and chickens as recipients. The sample was drawn from peri-domestic households in a single ecological zone, so generalisation to other climates or to commercial poultry systems should be cautious. Twelve B1-positive samples could not be fully genotyped, which may have biased genotype frequencies if those samples were systematically different. No mouse or cat bioassay was performed, so viable parasites were not isolated and virulence was not tested. Finally, human data were not collected, so the direct link to human toxoplasmosis in the same households remains inferential and should be tested in future paired human–animal studies.

Future research should (i) expand sampling to multiple seasons and localities to test for seasonal variation in oocyst survival and seroprevalence, (ii) include faecal oocyst detection in cats by microscopy and PCR to directly measure shedding, (iii) add human serology and risk-factor questionnaires in the same households to quantify zoonotic spillover, (iv) apply microsatellite typing or whole-genome sequencing to resolve atypical genotypes, and (v) evaluate low-cost

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interventions — such as keeping chickens in fenced runs, daily litter management, and community education — in a controlled trial. Such work would strengthen the evidence base for locally adapted, One Health control of toxoplasmosis.

5. CONCLUSION

Toxoplasma gondii is common in free-range chickens (35.6% seroprevalence) and domestic cats (41.0%) sharing peri-domestic environments. B1 PCR confirmed parasite DNA in 59.4% of seropositive chickens and 46.3% of seropositive cats, and multilocus PCR-RFLP genotyping of 45 isolates showed a predominance of Type II (64.4%), with Type III (22.2%) and atypical recombinant genotypes (13.3%) also present. Free-range chickens are reliable bio-indicators of environmental oocyst contamination shed by domestic cats, and the genotype diversity observed underscores the need for continued surveillance. Public health education on thorough meat cooking, safe handling of cat litter and soil, and feline hygiene is warranted to minimise human toxoplasmosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Mei-Ling Chen conceived the study, supervised laboratory work and drafted the manuscript. Hiroshi Takahashi performed molecular genotyping and data analysis. Ji-Hoon Park coordinated field sampling and serology. All authors reviewed and approved the final manuscript.

ETHICAL APPROVAL

Animal handling and sampling were conducted in accordance with institutional animal-care guidelines, with owner consent. No animals were killed solely for this study.

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