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Serological and Molecular Diagnosis of Toxoplasmosis Among Female Livestock Farmers in Kongo Central, Democratic Republic of the Congo

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ABSTRACT

Introduction

Toxoplasmosis represents a major public health concern due to its significant contribution to neonatal morbidity, mortality, and congenital complications. Diagnostic algorithms routinely rely on the detection of anti-*Toxoplasma gondii* antibodies (IgM/IgG) and molecular techniques, such as polymerase chain reaction (PCR).

Purpose

To evaluate and compare the performance of serological and molecular diagnostic methods for detecting *Toxoplasma gondii* infection among female livestock farmers in the Cataractes District, Kongo Central Province, Democratic Republic of the Congo.

Methods

An analytical cross-sectional study was conducted between November 2023 and February 2024 among 398 female pig farmers aged ≥ 18 years residing in the Cataractes District. Serological screening was performed by measuring anti-*T. gondii* IgM antibodies via the VIDAS Enzyme-Linked Fluorescent Assay (ELFA). Genomic DNA was extracted using the QIAGEN DNA extraction kit, and conventional PCR was conducted targeting the *T. gondii* B1 gene. PCR was utilized as the reference standard. Statistical analysis was performed using R software (version 4.3.2).

Results

Among the 398 participants, 63 (15.83%) were positive for anti-*T. gondii* IgM antibodies, and 74 (18.59%) tested positive by PCR. Mbanza-Ngungu Territory demonstrated high positivity, with 22 (5.53%) IgM-positive and 25 (6.28%) PCR-positive participants. Cross-tabulation revealed 56 true positives, 317 true negatives, 7 false positives (1.76%), and 18 false negatives (4.52%). A highly significant association was observed between IgM serology and PCR ($\chi^2 = 238.87$, df = 1, $p < .001$; Fisher's exact test $p < .001$; Odds Ratio [OR] = 135.61, 95% CI [52.81, 348.12]). Using PCR as the reference standard, IgM serology exhibited a sensitivity of 75.68% (95% CI [64.79, 84.02]), specificity of 97.84% (95% CI [95.61, 98.95]), overall accuracy of 93.72% (95% CI [90.89, 95.71]), positive predictive value (PPV) of 88.89% (95% CI [78.80, 94.51]), negative predictive value (NPV) of 94.63% (95% CI [91.67, 96.57]), and a Cohen's kappa coefficient (κ) of 0.7799.

Conclusion

Serological and molecular assays show strong diagnostic concordance and should be deployed as complementary tools for evaluating toxoplasmosis in occupationally exposed populations. Incorporating molecular screening into routine epidemiological surveillance will enhance early detection and targeted intervention strategies.

INTRODUCTION

Toxoplasmosis is a ubiquitous, cosmopolitan zoonotic infection caused by *Toxoplasma gondii* (*T. gondii*), an obligate intracellular protozoan parasite of the phylum Apicomplexa that infects humans and a broad range of warm-blooded animals (Kim et al., 2024; Wesołowski et al., 2023). It remains a substantial public health burden globally due to its association with neonatal morbidity, mortality, congenital malformations, and severe neurological sequelae in immunocompromised individuals (Hamaichat, 2020).

Diagnosis of toxoplasmosis relies primarily on serological detection of specific anti-*T. gondii* antibodies (IgM and IgG) (Souza et al., 2023) or direct parasite detection in peripheral blood, cerebrospinal fluid, bronchoalveolar lavage fluid, or tissue specimens using polymerase chain reaction (PCR) assays (Hamaichat, 2020). While serological assays are sensitive, cost-effective, and adaptable to automated systems, they cannot consistently differentiate acute infection from persistent antibody titers or past immunity. Additionally, serological testing may suffer from non-specific cross-reactivity with other parasitic pathogens (Bongenya et al., 2020). Conversely, molecular biology techniques have advanced infectious disease diagnostics by enabling direct nucleic acid detection irrespective of host immune status or clinical stage (Fadel et al., 2025; Bongenya et al., 2020). PCR assays provide high diagnostic sensitivity and specificity, allowing precise identification of opportunistic pathogens, including *T. gondii* (Fadel et al., 2025).

Toxoplasma gondii infects approximately one-third of the global human population alongside various domestic and wild intermediate host species, with felids acting as definitive hosts (Abdallah et al., 2020; Ferra et al., 2020). Human transmission occurs predominantly via consumption of raw or undercooked meat containing tissue cysts, ingestion of water or produce contaminated with sporulated oocysts, or direct environmental exposure to feline feces. Following ingestion, tachyzoites rapidly disseminate hematogenously before being contained by host cell-mediated immune responses (Th1). However, complete clearance is rarely achieved, leading to chronic, latent infection characterized by tissue cysts in skeletal muscle, the central nervous system, and ocular tissues (Gelaw et al., 2024). Environmental persistence of oocysts is enhanced in humid tropical climates, where high ambient humidity and inadequate hygiene practices facilitate transmission (Souza et al., 2023).

In Africa, seroprevalence rates for toxoplasmosis vary widely, ranging from 30% to over 80% depending on geographical zone, dietary practices, and environmental conditions. The highest regional prevalence has been documented in Central Africa (54.0%), contrasting with lower rates in West Africa (4.1%) and country-level estimates ranging from 2.6% in Namibia to 80.3% in the Republic of the Congo (Gelaw et al., 2024). Major risk factors include felid contact, handling raw meat, and agricultural soil exposure.

In the Democratic Republic of the Congo (DRC), empirical comparisons between serological and molecular diagnostic utilities remain sparse, particularly among occupationally exposed agricultural cohorts. Restricted availability of molecular infrastructure in peripheral health centers hampers accurate diagnostic resolution and disease surveillance. Previous investigations in Kinshasa and Bukavu demonstrated seroprevalence rates between 40% and 80% (Furaha et al., 2023; Mbo Duka et al., 2026). Female pig farmers represent a highly exposed subgroup due to frequent handling of livestock, soil contact, and exposure to potential oocyst-contaminated environments. This study aimed to evaluate and compare the diagnostic performance of anti-*T. gondii* IgM serology and PCR among female livestock farmers in the Cataractes District, Kongo Central Province.

METHODS

Study Site

This study was conducted across public referral centers in the Cataractes District, situated within Kongo Central Province, DRC (east of Matadi, on the left bank of the Congo River). The district covers an area of 23,481 km² with an estimated population of 1,436,642 inhabitants (density: 61.18 inhabitants/km²). The Cataractes District was selected due to its high density of veterinary settlements, widespread backyard pig farming, and favorable tropical climate for oocyst survival. According to the Köppen–Geiger climate classification, the region experiences a tropical savanna climate (*Aw*) with a mean

annual temperature of 23.7°C and annual precipitation averaging 781.6 mm. The district comprises three administrative territories: Mbanza-Ngungu, Luozi, and Songololo.

Study Design and Period

An analytical cross-sectional study was executed between November 2023 and February 2024 to evaluate serological (VIDAS ELFA) and molecular (PCR) diagnostic performance for toxoplasmosis.

Study Population and Sampling Procedure

A convenience sample of 398 female pig farmers aged ≥ 18 years residing in the Cataractes District was enrolled. Given the limited prior epidemiological baseline data for this specific occupational cohort, all eligible women meeting inclusion criteria during the enrollment window were invited to participate to maximize statistical power.

A. **Inclusion Criteria:** Female sex, age ≥ 18 years, active involvement in pig farming, continuous residence in Cataractes District during the study timeframe, and provision of written informed consent.

B. **Exclusion Criteria:** Refusal to grant informed consent, age < 18 years, or temporary residence status.

Data Collection and Laboratory Procedures

Socio-demographic data and risk factor profiles were collected using a structured, pre-tested questionnaire validated in the Lukala and Bas-Fleuve districts prior to full deployment.

i. **Serological Analysis:** Venous blood samples were collected, and serum was isolated for anti-*T. gondii* IgM antibody testing using the automated VIDAS® ELFA system (bioMérieux, Marcy-l'Étoile, France) according to manufacturer instructions. Cut-off thresholds were applied as follows: Index < 0.55 (Negative); 0.55 ≤ Index < 0.65 (Equivocal); Index ≥ 0.65 (Positive).

ii. **Molecular Analysis:** Genomic DNA was extracted from leukocyte fractions using the QIAGEN DNA Mini Kit (QIAGEN, Hilden, Germany) following manufacturer protocols and stored at -20^{0}C}. Conventional PCR amplification targeting the high-copy *T. gondii* B1 gene (producing a 529-bp amplified product or specific B1 target fragment) was performed using specified primer sets (Grigg & Boothroyd, 2001) in a 25 µL final reaction volume.

Table 1:
Primer Specifications for Toxoplasma gondii B1 Gene PCR Amplification

Target Gene	Primer Name	Sequence (5' → 3')	Product Size
B1	Toxo F2	CCGGGCAAGAAAATGAGAT	~529 bp
	Toxo R2	CATGGTTTGCACTTTTGTGG	

Note. F2: Forward primer; R2: Reverse primer; PCR: Polymerase chain reaction.

Table 2:
Thermal Cycling Conditions for Toxoplasma gondii Amplification

Reaction Step	Temperature (°C)	Duration	Cycles
Initial Denaturation	94	3 min	1
Denaturation	94	30 s	30
Annealing	59	30 s	
Extension	72	5 min	
Final Extension	72	1 min	1
Hold	4	∞	–

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using R software (version 4.3.2). Categorical variables were summarized as counts and percentages. Associations between serological and molecular testing were evaluated using Pearson's chi-square test or Fisher's exact test when expected cell counts fell below 5. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Diagnostic performance parameters—including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall diagnostic accuracy, and Cohen's kappa (κ) coefficient—were calculated using PCR as the reference standard. Statistical significance was set at α = .05.

Ethical Considerations

Ethical approval was granted by the Ethics Committee of the School of Public Health, University of Kinshasa (Approval No. ESP/CE/36/2024). Institutional authorization was obtained from the Central Veterinary Laboratory of Kinshasa. Written informed consent was obtained from all participants prior to enrollment. Anonymity was preserved using unique study identification codes.

RESULTS

A total of 398 female pig farmers were evaluated. Overall serological screening for anti-*T. gondii* IgM antibodies identified 63 positive cases (15.83%) and 335 negative

cases (84.17%) based on comparative cross-tabulation. PCR diagnostic testing identified 74 positive cases (18.59%) and 324 negative cases (81.41%) **(Table 3)**.

Mbanza-Ngungu Territory demonstrated the highest burden across administrative subdivisions, recording 22 IgM-positive (5.53%) and 25 PCR-positive (6.28%) cases. At the sector level, Luozi sector recorded 24 IgM-positive (6.03%) and 22 PCR-positive (5.53%) cases **(Table 4)**.

Table 3:
Overall Serological and Molecular Diagnostic Results Among Female Pig Farmers (N = 398)

Diagnostic Assay	Classification	Frequency (n)	Percentage (%)
IgM Serology	Positive	63	15.83
	Negative	335	84.17
	Total	**398**	**100.00**
PCR Assay	Positive	74	18.59
	Negative	324	81.41
	Total	**398**	**100.00**

Table 4:
Distribution of Serological (IgM) and Molecular (PCR) Results Across Territories and Sectors

Geographic Unit Territory	IgM+ (n)	IgM- (n)	PCR+ (n)	PCR- (n)
Songololo	9	132	13	128
Luozi	2	74	6	74
Mbanza-Ngungu	22	123	25	120
Sector				
Kimpese	6	92	12	86
Kivunda	8	35	14	29
Luima	3	40	1	42
Luozi	24	45	22	47
Mbuku	5	67	11	61
Nsona Nkulu	17	56	14	59

Note. Data represent raw counts per administrative division.

Evaluating diagnostic concordance using PCR as the reference standard revealed 56 true positive (TP; 14.07%), 317 true negative (TN; 79.64%), 7 false positive (FP; 1.76%), and 18 false negative (FN; 4.52%) results **(Table 5)**. The association between IgM serology and PCR was statistically significant ($\chi^2 = 238.87$, df = 1, $p < .001$; Fisher's exact test $p < .001$; OR = 135.61, 95% CI [52.81, 348.12]).

Table 5:
Contingency Table Comparing IgM Serology Against PCR Reference Standard

IgM Serology	PCR Positive (n)	PCR Negative (n)	Total (n)
IgM Positive	56 (TP)	7 (FP)	63
IgM Negative	18 (FN)	317 (TN)	335
Total	**74**	**324**	**398**

Note. TP: True Positive; FP: False Positive; FN: False Negative; TN: True Negative. $\chi^2 = 238.87$, df = 1, $p < .001$; OR = 135.61.

Diagnostic performance parameters of IgM serology compared to PCR demonstrated high specificity (97.84%) and NPV (94.63%), with moderate sensitivity (75.68%) and an overall diagnostic accuracy of 93.72%. Inter-rater agreement yielded a Cohen's kappa coefficient (κ) of 0.7799, indicating substantial agreement **(Table 6)**.

Table 6:
Diagnostic Performance Metrics of IgM Serology Relative to PCR Reference Standard

Performance Indicator	Value	Percentage (%)	95% Confidence Interval
Sensitivity	0.7568	75.68	[64.79, 84.02]
Specificity	0.9784	97.84	[95.61, 98.95]
Positive Predictive Value (PPV)	0.8889	88.89	[78.80, 94.51]
Negative Predictive Value (NPV)	0.9463	94.63	[91.67, 96.57]
Overall Accuracy	0.9372	93.72	[90.89, 95.71]
Balanced Accuracy	0.8676	86.76	—
Apparent Prevalence (IgM)	0.1583	15.83	—
True Prevalence (PCR)	0.1859	18.59	—
Cohen's Kappa (κ)	0.7799	—	—
McNemar's Test p-value	0.0455	—	—

DISCUSSION

This study presents a comparative diagnostic evaluation of serological (ELFA IgM) and molecular (PCR) techniques for detecting *Toxoplasma gondii* infection among female livestock farmers in Cataractes District, DRC.

The observed molecular prevalence (18.59%) exceeded IgM seroprevalence (15.83%). This difference aligns with established literature emphasizing the superior analytical sensitivity of molecular targets over antibody detection, particularly during early infection prior to robust seroconversion or in individuals with muted humoral responses (Hamid et al., 2024). High positivity in agricultural hubs such as Mbanza-Ngungu and Luozi underscores localized exposure risks tied to swine management, soil contact, and variable sanitation infrastructure.

The identification of 7 false-positive IgM results (1.76%) highlights the known vulnerability of IgM immunoassay formats to non-specific cross-reactivity, autoimmune interference, or lingering IgM persistence months after initial resolution (Denis et al., 2023). Conversely, 18 false-negative IgM cases (4.52%) emphasize that relying solely on IgM assays may miss active parasitemias detected by DNA amplification. These discrepancies confirm that

serology alone can lead to diagnostic misclassification (Denis et al., 2023).

Statistical analysis demonstrated a strong association between the two modalities (OR = 135.61, p < .001). Individuals testing positive by IgM serology exhibited over 135 times higher odds of PCR positivity compared to IgM-negative individuals. Combined with substantial inter-test agreement (κ = 0.7799), these findings confirm strong diagnostic concordance while supporting the clinical value of combined testing algorithms (Barbosa de Souza et al., 2023).

The diagnostic parameters observed in this setting—specificity of 97.84% and sensitivity of 75.68%—align with findings from gestational toxoplasmosis cohorts in Brazil, where molecular methods demonstrated 100% specificity and 60% sensitivity relative to clinical parameters (Murata et al., 2017). Furthermore, these metrics mirror global meta-analytic estimates of molecular diagnostic performance for toxoplasmosis, which report pooled specificities of 96.2% and moderate pooled sensitivities (66.0%), confirming PCR as a highly specific confirmatory assay (Suprianto et al., 2026).

Limitations

1. **Study Design & Sampling:** The cross-sectional design and non-probability convenience sampling restrict causal inferences regarding environmental risk factors and limit direct generalizability to broader national populations.
2. **Serological Scope:** Diagnostic serology was limited to anti-*T. gondii* IgM antibody testing. IgG serology and IgG avidity testing were not performed, preventing clear differentiation between hyperacute, subacute, and chronic persistent infections.
3. **Assay Comparisons:** Neither real-time quantitative PCR (qPCR) nor nested PCR formats were evaluated against conventional PCR, and formal cost-effectiveness analyses were not performed to assess local economic feasibility.

Recommendations

a) **Ministry of Health:** Integrate toxoplasmosis into national epidemiological disease surveillance framework guidelines, expand molecular diagnostic capacities (PCR) in regional reference laboratories, and establish standardized clinical management algorithms for high-risk occupational cohorts.

b) **Infectious Disease Control Programs:** Implement community-targeted health education campaigns addressing hygiene and transmission risks among agricultural workers, while supplying peripheral diagnostic centers with validated diagnostic kits.

c) **Healthcare Facilities:** Adopt integrated diagnostic protocols combining serological screening with molecular confirmation where feasible, particularly for exposed cohorts and pregnant women.

d) **Research Community:** Conduct longitudinal prospective studies utilizing probabilistic sampling, incorporate IgG avidity and real-time PCR platforms, and execute molecular genotyping of local *T. gondii* isolates.

e) **Female Livestock Farmers:** Adhere to targeted biosecurity measures, including regular hand hygiene following animal handling or soil contact, use of protective footwear, consumption of treated water, and thorough cooking of meat products.

Abbreviations:

i. **df:** Degrees of Freedom;
ii. **ELFA:** Enzyme-Linked Fluorescent Assay;
iii. **FN:** False Negatives;
iv. **FP:** False Positives;
v. **IgM:** Immunoglobulin M;
vi. **NPV:** Negative Predictive Value;
vii. **OR:** Odds Ratio;
viii. **PCR:** Polymerase Chain Reaction;
ix. **PPV:** Positive Predictive Value;
x. **TN:** True Negatives;
xi. **TP:** True Positives.

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Use of Artificial Intelligence (AI) Declaration: No artificial intelligence tools were used in the generation or interpretation of the findings of this study.

Authors' Contributions:

i. **CLT:** Conceptualization, methodology, field sampling, laboratory execution, manuscript drafting.
ii. **GIB:** Conceptualization, statistical analysis, data interpretation, manuscript writing and editing.
iii. **LOL:** Methodological support, data curation, critical manuscript review.
iv. **ENK:** Supervision, study validation, molecular expertise, statistical oversight, final manuscript approval.
v. All authors approved the final version.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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