RESEARCH ARTICLE

Molecular Diagnosis of Toxoplasma gondii in Free-Range Chickens (*Gallus gallus*) Sold at Fairs in the Metropolitan Region of Macapá–AP (Brazil)

Paulo Tarso Tavares Santana[1], Washington Luiz Assunção Pereira[2], Rafaelle Casseb Guimarães[3], Cintia Luana Pinheiro Santos[3], Juliana Vasconcelos Figueiredo[3], Roberta de Araújo Silva[3], Higo Gregório Silva Favacho[4], Ednaldo Silva Filho[3]*, Elizabeth Machado Barbosa[5]

[1]*Department of Animal Health Inspection, Health Surveillance Superintendence, Macapá, Brazil*
[2]*Laboratory of Veterinary Pathology, Federal Rural University of Amazon, Belém, Brazil*
[3]*Laboratory of Animal Biotechnology, Federal Rural University of Amazon, Belém, Brazil*
[4]*Department of Animal Science, Federal Institute of Amapá, Porto Grande, Brazil*
[5]*Department of Rural and Biological Sciences, Federal University of Amapá, Mazagão, Brazil*

*Corresponding Author: silva.filho@ufra.edu.br

Abstract: The informal commercialization of free-range poultry in open markets poses potential food safety risks regarding zoonotic pathogens. This investigation molecularly and histopathologically screened for *Toxoplasma gondii* in 36 free-range chickens acquired from 12 prominent markets in metropolitan Macapá, Amapá State. Paired heart and brain tissue samples from each animal were processed via conventional PCR and conventional histology. The pathogen was detected in 72.2% (n = 26) of the sampled population, driven by a high prevalence of concomitant tissue infection (58.3%, n = 21) and isolated brain positivity (13.9%, n = 5). Conversely, 27.8% (n = 10) of the flock tested negative, and microscopic examination yielded no evident bradyzoites or associated lesions. Sequenced amplicons were integrated into a genetic distance tree, demonstrating high identity and minimum evolutionary distance when clustered with a prominent group of 98 reference sequences. Spatial analysis confirmed environmental dispersion, with positive tissues originating from birds sold in 91.6% of the investigated commercial sites. The widespread presence of *T. gondii* in these retail outlets highlights a substantial risk of infection for local consumers. Our findings stress the urgency of establishing sanitary policies tailored to traditional markets in Amapá to control meat contamination and safeguard public health.

Keywords: Toxoplasmosis, *Gallus gallus,* Genetic Sequencing, Public Health

Received: 17-01-2026 | **Revised:** 03-04-2026 | **Accepted:** 23-04-2026 **| DOI:** 10.3844/ajavsp.2026.26.02.015

Introduction

Approximately one-third of the global human population is estimated to be seropositive for Toxoplasma gondii, characterizing toxoplasmosis as a cosmopolitan zoonosis [1]. The etiological agent, Toxoplasma gondii, is an obligate

DOI: 10.3844/ajavsp.2026.21.02.015

intracellular coccidian protozoan. Humans and homeothermic vertebrates serve as intermediate hosts, while domestic and wild felids act as definitive hosts [2].

In Brazil, toxoplasmosis is considered an endemic disease, with incidence varying between regions of the country. According to data from the Ministry of Health, 3,767 cases of toxoplasmosis were recorded in 2020 [3]. In Amapá State, between 2019 and 2023, 133 reported cases of toxoplasmosis in pregnant women were recorded, 112 of which were in the metropolitan area of Macapá (84.21%) [4].

In light of these findings, toxoplasmosis has emerged as a public health concern, particularly because its occurrence in food-producing animals raises important food safety issues [5]. Over the past four decades, chicken meat has exhibited the highest growth rate among all animal protein sources worldwide [6]. In the current scenario, Brazil leads the ranking of the world's largest exporter of chicken meat, contributing more than 35.98% of global exports (13,826 million tons), followed by the United States (23.24%) and the European Union (12.01%) as the three largest exporters [7].

In Brazil, extensive free-range chicken farming was encouraged by consumers' preference for natural products with less chemical residue, in addition to being a great alternative for family farming and the environment [8]. For the family, another alternative source of income was created; food and excrement can be used to fertilize fruit trees. With the establishment of poultry farms, families will be able to have better food and even generate profits. Furthermore, it is a completely sustainable activity, and, with appropriate management, there is no degradation of the environment. These animals are frequently found visiting open-air markets, and their consumption becomes more evident among the low-income population [9], who use these places as the main source for purchasing food.

The SARS-CoV-2 pandemic (2019) highlighted the need for improved health management in livestock production, given that poor practices may favor the emergence of viral, bacterial, and parasitic diseases with zoonotic potential [10]. [11] pointed out that the lack of health control and technical training in management has made the raising of free-range chickens (*Gallus gallus* domesticus) a risk to their commercialization, showing the need for more extensive health surveillance, generating the need for disease prevention and control strategies. These include food hygiene measures, control of the domestic animal population, and education of the population regarding the risks associated with zoonoses [2, 12].

In chickens, toxoplasmosis predominantly presents a subclinical form, with little clinical importance for this species [13]. Domestic chickens are considered important in the epidemiology of this disease, as they are sources of transmission for both humans and cats, favoring, through the latter, the spread of the disease by the elimination of oocysts into the environment [13].

Free-ranging chickens have been widely used to indirectly track environmental contamination with T. gondii oocysts due to their habit of feeding directly on the ground [14]. The detection of T. gondii in chickens sold in street markets may indicate failures in the sanitary inspection of animals, as well as provide information on the spread of the parasite in urban areas [13].

Studies related to the occurrence of T. gondii in production animals are still incipient, and in the search for identification and obtaining more effective results, molecular techniques aim to evaluate the presence of T. gondii in commercialized poultry, as well as contribute to health authorities to improve epidemiological surveillance and promote food safety [15].

Accordingly, this study utilizes Polymerase Chain Reaction (PCR) to screen for T. gondii in free-range chickens slaughtered across open-air markets in the Macapá metropolitan area (Amapá State), aiming to assess potential food safety risks for local consumers.

Materials and Methods

Sample Collection

Three free-range chickens (*G. gallus*) were purchased from twelve randomly selected market vendors from different locations in nine markets, totaling thirty-six animals. Two samples were taken from each slaughtered animal: one heart sample and one brain sample, individually packaged and properly identified with sample number, market location, and date (cooled and subsequently frozen), to perform seventy-two reactions for molecular diagnosis in the Microbiology/Immunology Laboratory of the Medical Department of the Federal University of Amapá. The selection of markets was based on their geographic distribution across the metropolitan region of Macapá, aiming to represent different urban areas and poultry supply sources. The number of sampled animals per market followed a standardized approach to ensure comparability among locations and to allow the detection of *T. gondii* circulation in the local food production chain.

In addition, one heart sample and one brain sample were collected for histopathological examinations from all free-range chickens. These samples were stored in a 10% formalin solution, then stained with hematoxylin-eosin and analyzed under a light microscope.

DNA Extraction and Molecular Analysis

Genomic DNA was extracted from 36 heart and 36 brain samples following the tissue protocol described by [16]. Next, the DNA was quantified in a BioDrop spectrophotometer (Thermo Scientific, Wilmington, UK) with absorbance at 260 nm, and purity was determined by the A260/230 and A260/280 ratios. Finally, the material was frozen and stored at -20°C.

After the extractions, the DNA samples were subjected to agarose gel electrophoresis (1.5%), in TAE 1X application buffer (40 mM Tris base; 20 mM glacial acetic acid; 1 mM EDTA, pH 8.0), using 2µL of extracted DNA with 2µL of the Blue/Gelred mix (1:1) at 90V, for approximately 30 minutes.

The B1 gene was selected as the molecular target due to its high copy number and proven sensitivity for the detection of *T. gondii* DNA in animal tissues. A 193-bp fragment of the *T. gondii* B1 gene was amplified using the primer pair forward 5'-GGAACTGCATCCGTTCTAGAG-3' and reverse 5'-TCTTTAAGCGTTGCTGGTC-3'. These primers were selected due to their proven specificity and frequent application in the molecular epidemiology of free-range chickens [17]. This target region has been widely recognized as reliable for detecting low parasite burdens in heart and brain tissues. Each PCR run incorporated a positive control (DNA extracted from a confirmed *T. gondii* isolate) and a negative control (ultrapure water in place of DNA template).

Each PCR reaction mixture (final volume 20 µL) contained 1.0 µL of DNA template, 10 µL of Taq DNA Polymerase 2x Master Mix (Amplion, Odense, Denmark), 0.8 µL of each primer (8 pmol/µL), 1.0 µL (5%) of Q-Solution (Qiagen, Brazil), and 6.4 µL of ultrapure water. Thermal cycling was conducted in a 2720 Thermal Cycler (Applied Biosystems) under the following conditions: initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30 s, 62°C for 30 s, and 72°C for 50 s; and a final extension at 72°C for 7 min.

The final PCR products were subjected to horizontal electrophoresis in 1.0% agarose gel at 100 V/90 mA for 60 minutes. The fragment sizes (bp) were visualized using an M-20 ultraviolet light transilluminator (UVP) using a 1000 bp molecular mass ladder standard as a reference.

The PCR products were purified using the PCR product purification kit in a column (Ludwig Biotec LTDA, Alvorada, RS, BR), following the manufacturer's recommendations, and were subjected to horizontal electrophoresis in 0.8% agarose gel with a current of 100 V/90mA for 45 minutes, and visualization will occur through the M-20 ultraviolet light transilluminator (UVP).

The purified products were sequenced by the Sanger method using the BigDye Terminator v 3.1 Cycle Sequencing Kit reagent (Invitrogen California/USA) in a final volume of 10 µL in an ABI 3500 Genetic Analyzer automated sequencer (Applied Biosystems). The sequences obtained were edited using the Finch TV version 1.4.0 program (Geospiza Research Team, USA), and the BLAST tool to contrast with the corresponding buffalo reference sequences entered in NCBI GenBank, and finally, they were aligned in the BioEdit 7.2 program using ClustalW multiple alignment.

Genetic Distance Tree Determination

The sequence described by the positive samples was aligned by the GenBank BLAST method with the sequences found with identity to the B1 gene of Toxoplasma gondii. Then, the elaboration of a genetic distance tree using the Neighbor Joining method was requested.

Statistical Analysis

The data were tabulated in spreadsheets, and the absolute and relative frequencies of positive and negative samples for Toxoplasma gondii were determined. A discordance analysis was established by the McNemar's test for the samples individually evaluated for the heart and brain tissues together. Statistical procedures were conducted using the SAS OnDemand computer program with a significance level of 5%.

DOI: 10.3844/ajavsp.2026.21.02.015

Results

In this investigation, molecular screening identified a high prevalence of Toxoplasma gondii DNA within the cardiac and cerebral tissues of free-range poultry retail-sold in traditional markets across metropolitan Macapá. The distribution data compiled in Tables 1 and 2 reveal that infected birds spanned the majority of the surveyed commercial sites, highlighting a pervasive environmental contamination and sustained exposure of the birds to the protozoan. PCR assays confirmed that 72.2% (26/36) of the evaluated chickens harbored the parasite. Concomitant tissue involvement (both heart and brain) was detected in 21 specimens (58.3%), whereas isolated cerebral positivity was restricted to five birds (13.9%); the remaining ten individuals (27.8%) exhibited no amplifiable DNA in either tissue matrix (Table 1).

It is worth highlighting that in the fairs with identifications from A to F, all of their analyzed animals were positive for *T. gondii*, while only fairs G (origin Padre Josino Community) and all of their animals were negative, as observed in Table 2.

Table 1: Association analysis of discordance of results between tissues according to individuals by McNemar's test

Heart	Brain	N (%)	Heart	Brain	N (%)
Positive	Positive	21 (58.3)	Positive	Negative	0 (0.0)
Negative	Negative	10 (27.8)	Negative	Positive	5 (13.9)
	Total	31 (86.1)		Total	5 (13.9)
	P-Value*	0.073		P-Value*	0.074

*P-Value = Probability by McNemar's Test

Table 2: Identification (ID) of positive and negative animals according to the purchase fair, origin, and municipalities

ID	Purchase fair	Origin of animals	Municipality	Results of animals	
				Positive	Negative
A	Mercado Central	Tracajatuba Community	Ferreira Gomes – AP	3	0
B	Perpetuo Socorro	Curralinho Community	Macapá – AP	3	0
C	Buritizal	Monte Alegre	Monte Alegre - PA	3	0
D	Mazagão	Ajudante Community	Mazagão – AP	3	0
E	Monte Castelo	Monte Castelo	Casa Grande - AP	3	0
F	Marabaixo	Goiabal	Macapá – AP	3	0
G	Novo Horizonte	Curiaú	Macapá – AP	3	0
		Maruanum		2	1
		Padre Josino Community		0	3
		Novo Horizonte		1	2
H	Santana	Cupixi Community	Porto Grande - AP	2	1
I	Pacoval	Ilha Redonda	Macapá – AP	0	3
		Total		26 (72,2%)	10 (27,8%)

DOI: 10.3844/ajavsp.2026.21.02.015

The samples, which were positive for Toxoplasma gondii, were detected by conventional PCR according to the B1 gene sequence, and were sequenced to prove the consistency of the reactions. The 193 bp amplicons were sequenced in both forward and reverse directions, and consensus sequences were generated and compared with the sequences deposited in GenBank. All sequences were confirmed for Toxoplasma gondii according to sequence identity ID: LN714499.1 (Figure 1).

TPA_asm: Toxoplasma gondii VEG, chromosome chrIX, complete genome
GenBank: LN714499.1

```
1   ggaactgcat ccgttcatga gtataagaaa aaaatgtggg aatgaaagag
51  acgctaatgt atttgcatag gttgcagtca ctgacgagct cccctctgct
101 ggcgaaaagt gaaattcatg agtatctgtg caactttggt gtattcgcag
151 attggtcgcc tgcaatcgat agttgaccac gaacgcttta aaga
```

Fig. 1: PCR product sequence showing 100% identity to GenBank accession LN714499.1

A genetic distance tree was created from 100 sequences available in GenBank. We can observe three sequences that form distinct groups and one group formed with the minimum distance between 98 sequences with a high degree of identity, including the sequence determined in this work (Figure 2).

Fig. 2: Genetic distance trees of the animals tested

The topological arrangement of the genetic distance tree, constructed from 100 public GenBank sequences, highlighted distinct evolutionary paths (Figure 2). While three sequences were segregated into individual branches, a substantial cluster was established based on minimal genetic distance among 98 entries. This high-identity group included the specific sequence resolved in this investigation.

Discussion

The use of the Polymerase Chain Reaction (PCR) is a major advance in the diagnosis of *T. gondii* infection. Several studies using the technique demonstrated greater accuracy, sensitivity, and specificity of the PCR technique when compared to other traditional methodologies [18]. In addition, the diagnosis can be confirmed in one day, with a greater possibility of detecting small amounts of target DNA and potentially providing an alternative sensitive diagnostic tool [19].

Initially developed, Polymerase Chain Reaction (PCR) assays for *T. gondii* detection established a baseline for subsequent investigations by [2, 20, 21]. These authors successfully employed the technique to identify parasitic DNA across diverse biological matrices, particularly cerebral and cardiac tissues. In this context, the molecular identification of *T. gondii* underscores active parasitic circulation and reflects substantial environmental contamination within the studied region [22]. To achieve this, the current study targeted a 193-bp amplicon of the repetitive B1 gene, an archive marker widely exploited for diagnostic tracking in tissue samples. While other loci, such as P30 and 18S rDNA, are also frequently utilized [17, 23, 24], the B1 region remains highly effective for localized screening.

Among the genetic markers available for *T. gondii* detection via PCR, the B1 gene region is frequently chosen due to its high degree of sequence conservation across different parasite strains [25]. Furthermore, the specificity of this target has

DOI: 10.3844/ajavsp.2026.21.02.015

already been proven in studies using clinical samples [17, 26]. As brain and heart samples were used in this study to detect the parasite, the B1 gene was used as the target region in order to obtain a better amplification result.

Spatial assessment across nine investigated markets in metropolitan Amapá State demonstrated a 91.6% positivity rate among the commercialized poultry flock. This prevalence surpasses the findings of [27], who detected *T. gondii* DNA in 80% (16/20) of analyzed markets, thereby confirming a broader dissemination of the protozoan throughout different urban zones. Regarding individual animal data, 72.2% (26/36) of the free-range chickens harbored the parasite, driven by concurrent brain and heart infection in 58.3% (n = 21) of the cohort, while single neurological positivity was observed in 13.9% (n = 5). Interestingly, while [28] indicated a higher parasitic tropism toward muscular tissues-including the heart-over the brain, our molecular assays successfully amplified *T. gondii* DNA within cerebral tissues across all positive specimens. Furthermore, [27] reported a clear correspondence between pooled cardiac and cerebral positive samples, which contrasts with the independent tissue distribution patterns observed in the present individual analysis.

When comparing the PCR technique with serology, [27] analyzed the frequency of seropositive birds and positive PCR results in the population studied in Goiânia-GO. Of the 80 birds, 70% of the positive results were higher in PCR, while serology by MAD was 38.75% serology titer. This result has also been proven by [17].

Seronegative birds may yield detectable parasite DNA by PCR, whereas seropositive individuals with high antibody titers can test negative by molecular methods, as demonstrated by [29]. This fact can be explained by the absence of parasite cysts in the analyzed fragment, showing the importance of increasing the number of samples researched.

Global epidemiological data reveal highly variable rates of *T. gondii* exposure in poultry. For instance, investigations utilizing the Modified Agglutination Test (MAT) or enzyme-linked immunosorbent assay (ELISA) coupled with histopathology and immunohistochemistry (IHC) demonstrated a lower prevalence in Egypt (8.5%; [30]) and China (7.26%; [31]), whereas a higher and fluctuating seroprevalence was documented across Caribbean nations such as Antigua and Barbuda (20.5%), Dominica (38.2%), and Trinidad (17.1%) by [32]. European studies, such as the one conducted by [28] in Austria, recorded a 36.3% seroprevalence in backyard flocks, which aligns closely with the 46.4% occurrence observed by [33] in the Brazilian states of Pará and Rio Grande do Sul.

A consensus among these researchers indicates that *T. gondii* seropositivity is heavily dictated by herd management, with extensive or free-range systems significantly increasing bird susceptibility. This management dynamic was verified by [34], who reported an 11% seroprevalence in free-range chickens compared to just 5% in confined ones. Similarly, [35 noted that infection rates reached 67.14% in free-ranging birds but dropped to 41% on intensive farms. The impact of the production framework is further illustrated by [36], who observed a lower parasite occurrence in short-cycle commercial broilers (12%) than in long-lived laying hens (33%)."

The PCR technique proved to be viable for detecting *T. gondii* DNA in primary samples from free-range birds, however [27] states that a pool of samples from the viscera to be analyzed may be more efficient, since the number of cysts may be small and there is not always enough DNA for detection or there may be no DNA in that fragment analyzed, this being an option.

This variation was evidenced by the previously mentioned authors regarding positivity, when analyzing different fragments of the same tissue, with 25 (31.25%), 15 (18.75%) and seven (8.75%) being detected in the analysis of fragments 1, 2 and 3, respectively, from part of the brain tissue, showing the importance of extracting more than one fragment from the same viscera, as it increases the chance of detecting the parasite's DNA in the sample, as previously reported by [20], who obtained 25% more positive samples in PCR from tissues of free-range chickens when using triplicate extraction. [37] used homogenized tissues, and the resulting samples were analyzed by Polymerase Chain Reaction (PCR), which allowed the detection of the parasite's DNA in 64%.

It is worth highlighting that geographic location can influence the seroprevalence of *Toxoplasma gondii* in animals in extensive farming systems, making these birds considered sentinels of environmental contamination, signaling the risk to public health [38], which was evidenced in this study, since the animals analyzed in this research come from small extensive farms in communities located in the Amapá and Pará States.

Regarding the nonspecific myocardial changes observed in four birds with chronic myocarditis, the lack of overt clinical signs aligns with classic descriptions by [2, 14, 33], who emphasize that avian toxoplasmosis predominantly manifests in a subclinical form. Given its limited clinical impact on this host species, these inflammatory lesions cannot be conclusively attributed to *T. gondii* infection. Nevertheless, other organic systems can be affected; for instance, [39, 40] identified distinct histomorphometric and histochemical alterations within the duodenal and ileal walls of infected poultry. Furthermore, [41] provided photomicrographic evidence of a tissue cyst embedded in the intestinal submucosa of a chicken infected with a *T. gondii* genotype II strain. In the present study, the failure to visualize tissue cysts microscopically is likely tied to a low parasite burden in the sampled fragments, coupled with the cyclical dynamics of the infection. This highlights why molecular assays like PCR offer far superior diagnostic sensitivity for detecting protozoan DNA compared to conventional histopathology.

Conclusion

The results of this study demonstrate a high incidence of *Toxoplasma gondii* in brain and heart samples from chickens commercialized in open-air markets in the metropolitan region of Macapá. These findings indicate that these animals, widely consumed by the local population, may represent an important source of human infection, particularly when poultry meat is improperly handled or insufficiently cooked.

The detection of the parasite in tissues with consumption potential highlights the epidemiological relevance of *T. gondii* in the context of food safety and public health, especially in regions characterized by informal marketing systems and limited hygienic and sanitary practices. Furthermore, the high frequency of infected animals suggests extensive circulation of the parasite in the environment and in poultry production systems.

Therefore, this study contributes to the understanding of toxoplasmosis as a foodborne zoonosis in the Amazon region and emphasizes the need for educational actions aimed at informing the population about proper handling and thorough cooking of poultry meat, as well as the adoption of good hygiene practices. In addition, the findings reinforce the importance of strengthening sanitary surveillance and conducting further studies to assess the risk of parasite transmission to consumers.

Acknowledgment

The authors express their gratitude to the Federal Rural University of the Amazon (UFRA) for providing the laboratory infrastructure utilized in this study, which was paramount to the execution and technical quality of the generated results.

Funding Information

This study did not receive funding from public, commercial, or non-profit funding agencies. All costs related to the execution of the study were fully covered by the authors.

Authors Contributions

Paulo Tarso Tavares Santana and Elizabeth Machado Barbosa: Developed the project, collected samples, performed data analysis.
Washington Luiz Assunção Pereira and Rafaelle Casseb Guimarães: Developed the project, performed data analysis.
Ednaldo Silva Filho: Developed the project, performed data analysis, and performed the statistical analysis on the variable data.
Cintia Luana Pinheiro Santos, Juliana Vasconcelos Figueiredo and Roberta de Araújo Silva: Performed data analysis.
Higo Gregório Silva Favacho: Collected samples.
All authors read, reviewed, and approved the structure and writing of the manuscript. There is no conflict of interest among authors.

Ethics

The experimental protocols of this study received formal clearance from the Ethics Committee on the Use of Animals (CEUA) at UFRA on November 30, 2023, under protocol number 6216180923 (ID 000645). Furthermore, all procedures strictly adhered to the animal welfare guidelines mandated by the National Council for the Control of Animal Experimentation (NCCAE) of Brazil.

References

1. Zhou, P., Chen, Z., Li, H.-L., Zheng, H., He, S., Lin, R.-Q., & Zhu, X.-Q. (2011). Toxoplasma gondii infection in humans in China. Parasites & Vectors, 4(1), 165. https://doi.org/10.1186/1756-3305-4-165
2. Dubey, J. P. (2010). Toxoplasma gondii infections in chickens (Gallus domesticus): prevalence, clinical disease, diagnosis and public health significance. Zoonoses and Public Health, 57(1), 60–73. https://doi.org/10.1111/j.1863-2378.2009.01274.x
3. Brazil. (2021). Toxoplasmosis. Portal Gov.Br. https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z/t/toxoplasmose
4. Carmo, E. L. do, Morais, R. dos A. P. B., Silva, A. V. da, Bichara, C. N. C., & Póvoa, M. M. (2025). A influência ambiental e a infecção pelo Toxoplasma gondii na Amazônia: contribuição do Instituto Evandro Chagas (Pará, Brasil). Revista Pan-Amazônica de Saúde, 16, e202501814.
5. Dubey, J. P., & 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
6. Talamini, D. J. D., Abreu, P. G. de, Codebella, A., & Bertol, T. M. (2023). Viabilidade econômica de diferentes sistemas tecnológicos de produção de frangos. Revista Tecnologia e Sociedade, 19(57), 330–349. https://doi.org/10.3895/rts.v19n57.17122
7. United States Department of Agriculture. (2024). Livestock and Products Semi-Annual.
8. Lourençoni, D., Faustino, A. C., Turco, S. H. N., Bonfim, O. C., Carolina, L., Silva, R. E., & Sá Silva Lins, A. C. (2022). Retraction to: Economic viability in free-range chicken production. Translational Animal Science, 6(2), 034. https://doi.org/10.1093/tas/txac034
9. Dias, A. O., Silva, L. M., Santos, R. P., Oliveira, J. F., & Souza, A. C. (2015). Consumption of poultry meat and eggs by the population of the Petrolina region. Extramuros – Revista de Extensão Da UNIVASF, 3(1), 128–134.
10. Sironi, M., Hasnain, S. E., Rosenthal, B., Phan, T. G., Luciani, F., Shaw, M.-A., Sallum, M. A. M., Mirhashemi, M. E., Morand, S., & González-Candelas, F. (2020). SARS-CoV-2 and COVID-19: A genetic, epidemiological, and evolutionary perspective. Genetics and Evolution, 84, 104384. https://doi.org/10.1016/j.meegid.2020.104384
11. McMullin, P. (2022). Infectious diseases in free-range compared to conventional poultry production. Avian Pathology, 51(5), 424–434. https://doi.org/10.1080/03079457.2022.2086448
12. Pappas, G., Roussos, N., & Falagas, M. E. (2009). Toxoplasmosis snapshots: Global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. International Journal for Parasitology, 39(12), 1385–1394. https://doi.org/10.1016/j.ijpara.2009.04.003
13. Dubey, J. P., Pena, H. F. de J., Cerqueira-Cézar, C. K., Murata, F. H. A., Kwok, O. C. H., Yang, Y.-R., Gennari, S. M., & Su, C. (2020). Epidemiologic significance of Toxoplasma gondii infections in chickens (Gallus domesticus): the past decade. Parasitology, 147(12), 1263–1289. https://doi.org/10.1017/S0031182020001134
14. Shwab, E. K., Zhu, X.-Q., Majumdar, D., Pena, H. F. de J., Gennari, S. M., Dubey, J. P., & Su, C. (2014). Geographical patterns of Toxoplasma gondii genetic diversity revealed by multilocus PCR-RFLP genotyping. In Parasitology (Vol. 141, Issue 4, pp. 453–461). https://doi.org/10.1017/S0031182013001844
15. Mahmood, D., & Nahhas, S. A. (2025). Serological and molecular detection of Toxoplasma gondii in chickens in southern Syria. Food and Waterborne Parasitology, 39, e00255. https://doi.org/10.1016/j.fawpar.2025.e00255
16. Pearson, H., & Stirling, D. (2003). DNA extraction from tissue. 226. https://doi.org/10.1385/1-59259-384-4:33
17. Holsback, L., Pena, H. F. de J., Ragozo, A., Lopes, E. G., Gennari, S. M., & Soares, R. M. (2012). Serologic and molecular diagnostic and bioassay in mice for detection of Toxoplasma gondii in free range chickens from Pantanal of Mato Grosso do Sul. Pesquisa Veterinária Brasileira, 32(8), 721–726. https://doi.org/10.1590/S0100-736X2012000800007
18. Dehkordi, F. S., Haghighi Borujeni, M. R., Rahimi, E., & Abdizadeh, R. (2013). Detection of Toxoplasma gondii in Raw Caprine, Ovine, Buffalo, Bovine, and Camel Milk Using Cell Cultivation, Cat Bioassay, Capture ELISA, and PCR Methods in Iran. In Foodborne Pathogens and Disease (Vol. 10, Issue 2, pp. 120–125). https://doi.org/10.1089/fpd.2012.1311
19. Sah, R. P., Talukder, M. H., Rahman, A. K. M. A., Alam, M. Z., & Ward, M. P. (2018). Seroprevalence of Toxoplasma gondii infection in ruminants in selected districts in Bangladesh. Veterinary Parasitology, Regional Studies and Reports, 11, 1–5. https://doi.org/10.1016/j.vprsr.2017.10.008
20. Holsback, L., Pena, H. F. de J., Bonapaz, R. dos S., Gennari, R. M., & Dubey, J. P. (2012). Serologic and molecular diagnosis and mouse bioassay for detection of Toxoplasma gondii in free-range chickens from Brazil. Pesquisa Veterinária Brasileira, 32(8), 721–726.
21. Pena, H. F. de J., Neto, S. V., Beltrame, M. A. A., Pereira, F. E. L., Gennari, S. M., & Soares, R. M. (2012). PCR-RFLP genotyping of Toxoplasma gondii from chickens in Brazil. Veterinary Parasitology, 192(1–3), 111–117.
22. Santos Silva, A. C. dos, Barros, L. D. de, Barros, V. M. C., Alcântara, A. M. de, Andrade, M. R., Garcia, J. L., Mota, R. A., & Nascimento Porto, W. J. (2020). Occurrence of Atypical and New Genotypes of Toxoplasma Gondii in Free-Range Chickens Intended for Human Consumption in Brazil. Acta Parasitologica, 65(3), 774–778. https://doi.org/10.2478/s11686-020-00194-2
23. Buchbinder, S., Blatz, R., & Christian Rodloff, A. (2003). Comparison of real-time PCR detection methods for B1 and P30 genes of Toxoplasma gondii. Diagnostic Microbiology and Infectious Disease, 45(4), 269–271. https://doi.org/10.1016/s0732-8893(02)00549-7
24. Tenter, A. M. (2009). Toxoplasma gondii in animals used for human consumption. Memórias Do Instituto Oswaldo Cruz, 104(2), 364–369.
25. Kompalic-Cristo, A., Britto, C., & Fernandes, O. (2005). Molecular diagnosis of toxoplasmosis: review. Jornal Brasileiro de Patologia e Medicina Laboratorial, 41(4), 229–235.
26. Wong, S.-Y., & Remington, J. S. (1994). Toxoplasmosis in pregnancy. Clinical Infectious Diseases, 18, 853–861. https://doi.org/10.1093/clinids/18.6.853
27. Neto, J. O. da S., Souza, A. M. de, Costa, R. F., Dubey, J. P., & Gennari, S. M. (2022). Serological and molecular investigation of Toxoplasma gondii in free-range chickens commercialized in Brazil. Brazilian Journal of Development, 8(2), 13705–13719.

28. Dubey, J. P., Edelhofer, R., Marcet, P., Vianna, M. C. B., Kwok, O. C. H., & Lehmann, T. (2005). Genetic and biologic characteristics of Toxoplasma gondii infections in free-range chickens from Austria. In Veterinary Parasitology (Vol. 133, Issue 4, pp. 299–306). https://doi.org/10.1016/j.vetpar.2005.06.006
29. Aigner, C. P., Silva, A. V. da, Sandrini, F., Osório, P. de S., Poiares, L., & Largura, A. (2010). Real-time PCR-based quantification of Toxoplasma gondii in tissue samples of serologically positive outdoor chickens. Memórias Do Instituto Oswaldo Cruz, 105(7), 935–937. https://doi.org/10.1590/s0074-02762010000700018
30. Rong, G.-Y., Zhou, H.-C., Hou, G.-L., & Zhang, X.-Z. (2014). Seroprevalence, risk factors and genotyping of Toxoplasma gondii in domestic geese (Anser domestica) in tropical China. Parasites & Vectors, 7(1), 459. https://doi.org/10.1186/s13071-014-0459-9
31. Cong, W., Huang, S.-Y., Zhou, D.-H., Xu, M.-J., Wu, S.-M., Yan, C., Zhao, Q., Song, H.-Q., & Zhu, X.-Q. (2012). First report of Toxoplasma gondii infection in market-sold adult chickens, ducks and pigeons in northwest China. Parasites & Vectors, 5(1), 110. https://doi.org/10.1186/1756-3305-5-110
32. Hamilton, C. M., Robins, R., Thomas, R., Oura, C. A. L., Oliveira, S. de, Villena, I., Innes, E. A., Katzer, F., & Kelly, P. J. (2019). Prevalence and genetic diversity of Toxoplasma gondii in free-ranging chickens from the Caribbean. Acta Parasitologica, 64(4), 738–744. https://doi.org/10.2478/s11686-019-00071-7
33. Dubey, J. P., Su, C., Oliveira, M. C., Gennari, R., Pena, H. F. de J., & Gennari, S. M. (2007). Biologic and genetic comparison of Toxoplasma gondii isolates in free-range chickens from Brazil. Veterinary Parasitology, 143(2), 182–188.
34. Yang, N.-Z., Mu, X., Yuan, Y., Lu, X.-X., & Zhang, X.-Z. (2012). Seroprevalence of Toxoplasma gondii in slaughtered poultry in China. Parasites & Vectors, 5(1), 237. https://doi.org/10.1186/1756-3305-5-237
35. Liu, X.-C., He, Y., Zhao, Q., Zhang, J., & Li, H. (2017). Detection of Toxoplasma gondii in chickens and soil of chicken farms in China. Infectious Diseases of Poverty, 6(1), 62.
36. Millar, P. R., Dubey, J. P., Ferreira, J. L., Oliveira, J. L., & Gennari, M. R. (2008). Importance of production animals in Toxoplasma gondii infection in Brazil. Semina: Ciências Agrárias, 29(3), 693–706. https://doi.org/10.5433/1679-0359.2008v29n3p693
37. Rezende, H. H. A., Silva, A. C. S., Dubey, J. P., & Gennari, S. M. (2021). Molecular characterization of Toxoplasma gondii isolates from free-range chickens. Brazilian Journal of Veterinary Parasitology, 30(2), 1–10. https://doi.org/10.1590/S1984-29612021029
38. Neto, J. O. da S., Azevedo, S. M., Lima, A. F., Silva, E. M., & Gennari, R. M. (2008). Prevalence and risk factors for anti-Toxoplasma gondii antibodies in goats from northeastern Brazil. Veterinary Parasitology, 156(3–4), 329–332.
39. Bonapaz, R. dos S., Hermes-Uliana, C., Santos, F. do N., Silva, A. V. da, Araújo, E. J. de A., & Sant'Ana, D. de M. G. (2010). Effects of infection with Toxoplasma gondii oocysts on the intestinal wall and the myenteric plexus of chicken (*Gallus gallus*). Pesquisa Veterinária Brasileira, 30(9), 787–792. https://doi.org/10.1590/s0100-736x2010000900013
40. Shiraishi, C. S., Azevedo, J. F. de, Silva, A. V. da, Sant'Ana, D. de M. G., & Araújo, E. J. de A. (2009). Análise morfométrica da parede intestinal e dinâmica de mucinas secretadas no íleo de frangos infectados por Toxoplasma gondii. Revista Ciência Rural, 39(8), 2146–2153. https://doi.org/10.1590/S0103-84782009000700030
41. Literák, I., & Hejlicek, K. (1993). Incidence of Toxoplasma gondii in domestic birds in the Czech Republic. Avian Pathology, 22(2), 275–281. https://doi.org/10.1080/03079459308418920