DOI: https://doi.org/10.61841/5a4j2a33 Publication URL: https://jarmhs.com/index.php/mhs/article/view/605

MOLECULAR DIAGNOSIS OF TOXOPLASMA GONDII AND SARCOCYSTIS HOMINIS IN SPUTUM SAMPLES FROM PATIENTS WITH RESPIRATORY DISEASES.

Marwa A. Hassan[1*], Muslim A.M. AL Tooma[2], Prof. Dr. Salwa A. Abduljaleel[3]

[1] *Department of Biology, College of Science, University of Misan, Amarah, Maysan, Iraq.*

[2] *Department of Pathological Analyses, College of Science, University of Basrah, Basrah, Iraq.*

[3] *Department of biology, faculty of science, University of Basrah, Basrah, Iraq.*

Corresponding Author:
marwaabdulhussein@uomisan.edu.iq

To Cite This Article: Marwa A. Hassa, Tooma, M. A. A., & Abduljaleel, P. S. A. (2026). MOLECULAR DIAGNOSIS OF TOXOPLASMA GONDII AND SARCOCYSTIS HOMINIS IN SPUTUM SAMPLES FROM PATIENTS WITH RESPIRATORY DISEASES. *Journal of Advanced Research in Medical and Health Science (ISSN 2208-2425)*, *12*(2), 43-47. https://doi.org/10.61841/5a4j2a33

ABSTRACT

Toxoplasma gondii and Sarcocystis hominis are worldwide zoonotic protozoan parasites of the phylum Apicomplexa, which represent a serious public health burden. Although pulmonary toxoplasmosis is primarily described in immunocompromised patients, molecular data regarding the detection of these parasites on sputum specimens are limited. Therefore, we used PCR to detect Toxoplasma gondii and Sarcocystis hominis DNA in sputum samples of patients with respiratory symptoms clinically compatible with pulmonary tuberculosis. A total of 30 sputum specimens for were collected from the patients who attending Chest and Respiratory Diseases Consulting Clinic in Maysan Governorate, Iraq from July-December/2025. The patients were similarly reported to have respiratory symptoms consistent with pulmonary tuberculosis, but were later found laboratory negative for tuberculosis on further investigation. Genomic DNA extraction was performed by Presto™ Rapid Extract PCR Kit and monoplex PCR assays were carried out using species primers. Molecular evidence thus proved that 8 of the thirty samples (26.7%) were Toxoplasma gondii positive giving a Sarcocystis hominis negative test result. These results suggest a possible association between respiratory manifestation of the disease and toxoplasmosis, as it was also observed in patients suspected to have pulmonary tuberculosis while the absence of Sarcocystis hominis detection might represent not only its narrow tissue tropism but also restricted features of transmission. PCR-based diagnosis of latent or falls parasitic diseases in sputum samples have high sensitivity and it might help to differentiate respiratory disease with pulmonary tuberculosis-like symptoms. These findings show the clinical importance of Toxoplasma gondii as a respiratory pathogen and suggest that molecular diagnostic methods are warranted to track this organism in endemic regions.

KEYWORDS: *Eucoccidiorida, Toxoplasma gondii, Sarcocystis hominis, PCR, Sputum samples.*

INTRODUCTION

Members of the phylum Apicomplexa are an ancient group of obligate intracellular protozoan parasites that represent a major cause human and veterinary diseases. *Toxoplasma gondii* and *Sarcocystis hominis* are two of the major zoonotic coccidia distributed all over the world with considerable importance to public health. These parasites illustrate a complex life cycle with tissue invasion, intracellular replication and potential systemic dissemination depending on immune status.

Toxoplasma gondii causes a parasitic disease that infects almost one third of the world population and is mainly transmitted through ingestion of cysts in undercooked or raw meat, oocysts from food, water and soil (Vilela & Feitosa, 2024). After intestinal invasion, it distributes hematogenously as tachyzoites and causes chronic infections in the brain, retina, skeletal muscles, lung and many other organs. Pulmonary toxoplasmosis, although less frequent than central nervous system disease has also been recognized in immunosuppressed patients and may present with interstitial pneumonitis, hacking cough, dyspnea and fever (Desoubeaux *et al*., 2016). Of note, recent molecular data has detected *T. gondii* DNA in respiratory association samples, notably sputum and bronchoscopy lung lavage fluid (a possible indication of pulmonary involvement or systemic dissemination) (Lass *et al*., 2017).

Moreover, *Sarcocystis hominis* is also a primarily zoonotic intestinal parasite and humans are infected by serving as a definitive host after the ingestion of raw or undercooked beef containing sarcocysts. Most intestinal infections are typically self-limited, but those that do present may be associated with abdominal pain, diarrhea and nausea (Fayer, 2004). Rhabdomyolysis linked to additional species of *Sarcocystis* has been demonstrated in the setting of systemic involvement including fever and myalgia, while there is no definitive evidence for isolated respiratory tract involvement with *S. hominis*. However, because a plethora of biologically well-characterized tissue-forming coccidia exists, transient detection or aberrant propagation cannot be ruled out (Harris *et al*., 2015)

The advances in molecular diagnosis have greatly enhanced detection within clinical samples and PCR allows for much higher sensitivity and specificity than conventional microscopy of protozoan parasites (Fitri *et al*., 2022). Such techniques have broadened our understanding of parasite distribution outside of classical infection sites, and revealed that parasitic DNA is detectable in atypical specimens at advanced stages of systemic infection. PCR-based diagnostics has remained a novel useful paradigm for infectious disease research (Ashraf *et al*., 2023; Pistone *et al*., 2026), supported by the advances in molecular epidemiology, that continue to expand our clinical relevance and capacity of pathogen detection from diverse clinical scenarios.

Despite these scientific advances, there are still very few data on the molecular detection of *T. gondii* and *S. hominis* in sputum samples from respiratory diseases patients. This is a critical information gap, particularly in endemic regions where access to diagnostic capabilities may be limited. Consequently, this study attempts to detect these parasite species from sputum specimens by polymerase chain reaction (PCR) and their prevalence and probable clinical significance in patients with respiratory symptoms. This work is significant because it will help clarify some of the difficulties associated with diagnosing atypical and extra-intestinal parasitic infections, while furthering novel perspectives regarding protozoan parasites as possible contributors to respiratory disease.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

In this study, we included a total of 30 sputum specimens from patients with suspected pulmonary tuberculosis patients with cough for more than two weeks, hemoptysis, chest tightness, shortness of breath combined with other symptoms such as fever and night sweats, weight loss. All specimens were collected and processed at the Chest and Respiratory Diseases Consulting Clinic of the Maysan Health Department, Maysan Governorate, from July 2025 to December 2025.

TISSUE DNA EXTRACTION

DNA extraction was performed on Eucoccidiorida species isolates using the Presto™ Rapid Extract PCR Kit (Geneaid, Taiwan). Then the DNA was subjected to monoplex PCR. The quality and the concentration of extracted DNA were assessed and quantified by Quantus™ Fluorometer (Promega, USA).

MOLECULAR ESTIMATION OF GENES USING PCR TECHNIQUE

The protocol was performed according to the manufacturer's instructions (Promega, USA).

PRIMERS

The specific primers were selected as shown in table (1) and synthesized by Promega (USA).

Table 1: Primers used in this study

NO.	Species	Types of primers	Primers	Primer sequences (5' → 3')	Product lengths	References
	Toxoplasma gondii	Nested	TgB1-vF1	TCAAGCAGCGTATTGTCGAG	194 bp	(Fallahi *et al*., 2014)
			TgB1-R1	CCGCAGCGACTTCTATCTCT		
			TgB1-F2	GGAACTGCATCCGTTCATGAG		
			TgB1-R2	TCTTTAAAGCGTTCGTGGTC		
			18SrRNAR	ATGAGAGACCTCACAGCCAAAC		
	Sarcocystis hominis	Conventional	COI-F	AATGTGGTGCGGTATGAACT	420 bp	(Oğuz & Değer, 2022)
			COI-R	GGCACCAACGAACATGGTA		

THE COMPONENT OF A PCR REACTION

All components of the PCR reaction for all genes are equal in type and size, except for the type of primer added, table (2) shows these components.

All components of the PCR reaction were used for target genes are equal in type and size, except for the type of primer detailed in table (2).

Table 2: The components of a PCR reaction

Component	Volume (µl)
GoTaq Green Master Mix (Promega)	12.5
Forward primer	1
Reverse primer	1
Nuclease-free water	4.5
gDNA	6
Total	25

THERMAL CYCLING CONDITIONS

All PCR components were assembled in Eppendorf PCR tube, then placed in the thermocycler (Eppendorf, Germany) and the right PCR cycling program parameter conditions were conducted as in table (3).

Table 3: Thermal Cycling Conditions

NO.	Genes	Temperature °C/ time					Cycles number
		Initial denaturation	Cycling conditions			Final extension	
			Denaturation	Annealing	Extension		
	B1 gene for *T. gondii*	94/5 min	94/1 min	55/2 min	72/1 min	72/10 min	30
	cox1 gene for *S. hominis*	95/3 min	95/1 min	58/1 min	72/30 sec	72/5 min	35

AGAROSE GEL AND ELECTROPHORESIS

Six microliters of amplified PCR product were loaded into the appropriate wells of the TAE agarose gel (1.5% (w/v)), with standard molecular weight of DNA ladder was loaded in one well and running using TBE 1X buffer was added to the electrophoresis tank, tray with agarose was immersed in electrophoresis tank. Electrophoreses run at 85 volts for 50 min, the gel stained with 2 µg/ml of ethidium bromide dye was visualized using a UV transilluminator.

ETHICAL APPROVAL

Ethical approval was granted by Maysan Health Directorate/the Chest and Respiratory Diseases Consulting Clinic. Patient data were handled confidentially, and samples were used solely for research purposes.

RESULTS

The samples obtained from individuals exhibiting suspected pulmonary tuberculosis were sent to the center by specialized physicians. The results confirmed they were not infected with the disease. However, when these samples were examined using PCR technique, it was found that 8 out of 30 patients were infected with toxoplasmosis (26.7%), while no samples showed infection with *Sarcocystis hominis*, as shown in the figure below for *Toxoplasma gondii*.

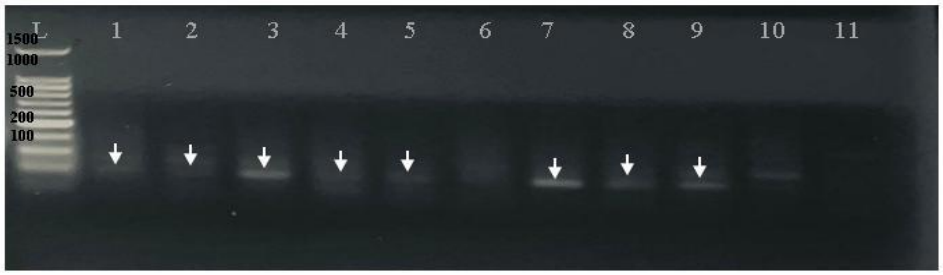

Figure 3-1: Ethidium bromide-stained Agarose Gel Electrophoresis (Second round) amplification of nested *B1* gene for *T. gondii*, Lane L DNA molecular size Ladder (100-1500) bp, Positive samples are marked with an arrow and include the following samples: (1, 2, 3, 4, 5, 7, 8 & 9).

DISCUSSION

Clinical experience shows that pulmonary Tuberculosis is suspected in just about all the serious coughs, but a substantial proportion turns out to not have tuberculosis. Therefore, clinicians frequently categorize them as allergic diseases or chronic hyper-sensitivity conditions and prescribe anti-allergic drugs. On the other hand, current study showed that a large number of patients who visited the Chest and Respiratory Diseases Consulting Clinic in Maysan Governorate with symptoms suggestive of pulmonary tuberculosis, who were laboratory proved to be TB-negative were infected by Toxoplasmosis. Importantly, it is recognized that *Toxoplasma gondii* can infect various tissues and organs throughout the body (Babekir *et al*., 2023).

The relatively high percentage of positive samples (26.7%) stands out as this could indicate possible respiratory transmission or pulmonary lesions smaller than those detectable by conventional imaging techniques (X-ray, ultrasound). This observation corroborates the role of Polymerase Chain Reaction being highly specific and sensitive as opposed to conventional diagnostics. Numerous studies have shown that PCR is able to identify minute amounts of parasitic DNA and can detect a variety of parasites during latent or low intensity infections (Robert-Gangneux & Dardé, 2012), but serological practices are not specific enough to define the precise infection site since they only imply previous exposure.

Detection of the parasite in sputum samples may be associated with coughing that is so severe that it ruptures tissue cysts and releases the parasite. Another potential explanation for this phenomenon is that extensive matting and adequate parasite burden allows the organism to be perpetually shed in sputum (Laibe *et al*., 2006). Our results corroborate previous studies suggesting that Toxoplasmosis may go unrecognized in many patients presenting with nonspecific symptoms, particularly in developing countries where exposure rates are high (Montoya & Rosso, 2005).

To exclude the possibility of contamination of sputum samples with saliva containing remnants of infected meat, human tissues previously confirmed to be infected with *Toxoplasma gondii* (as reported in the first study) were boiled for 15 minutes and then re-examined using PCR. The results were negative, indicating that contamination from food residues was unlikely. This is further supported by the common dietary practices in Iraq, where meat is typically cooked thoroughly for extended periods during food preparation (Li *et al*., 2026).

The present study did not detect any cases of *Sarcocystis hominis*. This may be attributed to the parasite's inability to cause pulmonary infection or its absence in lung tissues. The infection is also restricted by transmission routes, particularly the ingestion of undercooked beef (Fayer, 2004). On the other hand, *Toxoplasma gondii* is transmitted through three different transmission routes and its life cycle has more variety of infective stages than *Sarcocystis hominis*.

These findings highlight the importance of detecting latent parasitic infections using PCR techniques, and the need for diagnostic work-up when considering Toxoplasmosis as part of differential diagnosis with similar clinical presentations to tuberculosis. This likewise concurs with WHO hints (World Health Organization 2023) accentuating the requirement for exact determination and stressing that misdiagnoses ought to be kept away from utilizing current strategies.

REFERENCES

1. Ashraf, M. A., Mustafa, B. E., Wahaab, A., Batool, H., Ashraf, M., Said, M. B., ... & Stevenson, N. J. (2023). Conventional and molecular diagnosis of parasites. In *Parasitism and parasitic control in animals: strategies for the developing world* (pp. 56-72). GB: CABI. https://doi.org/10.1079/9781800621893.0004
2. Babekir, A., Mostafa, S., & Obeng-Gyasi, E. (2023). The Association of Toxoplasma gondii with the Combination of Cardiovascular Disease, Chronic Kidney Disease, or Chronic Liver Disease: A Preliminary Study. Medical Sciences, 11(4), 65. https://doi.org/10.3390/medsci11040065
3. Desoubeaux, G., Cabanne, É., Franck-Martel, C., Gombert, M., Gyan, E., Lissandre, S., ... & Chandenier, J. (2016). Pulmonary toxoplasmosis in immunocompromised patients with interstitial pneumonia: a single-centre prospective study assessing PCR-based diagnosis. *Journal of Clinical Pathology*, *69*(8), 726-730. https://doi.org/10.1136/jclinpath-2015-203385
4. Fayer, R. (2004).Sarcocystis spp. In human infections.*Clinical Microbiology Reviews*, 17(4), 894–902. https://doi.org/10.1128/CMR.17.4.894-902.2004

5. Fitri, L. E., Candradikusuma, D., Setia, Y. D., Wibawa, P. A., Iskandar, A., Winaris, N., & Pawestri, A. R. (2022). Diagnostic Methods of Common Intestinal Protozoa: Current and Future Immunological and Molecular Methods. *Tropical Medicine and Infectious Disease*, *7*(10), 253. https://doi.org/10.3390/tropicalmed7100253
6. Harris, V. C., Van Vugt, M., Aronica, E., De Bree, G. J., Stijnis, C., Goorhuis, A., & Grobusch, M. P. (2015). Human extraintestinal sarcocystosis: what we know, and what we don't know. *Current Infectious Disease Reports*, *17*(8), 42.
7. Laibe, S., Ranque, S., Curtillet, C., Faraut, F., Dumon, H., & Franck, J. (2006). Timely diagnosis of disseminated toxoplasmosis by sputum examination. *Journal of Clinical Microbiology*, 44(2), 646–648. https://doi.org/10.1128/JCM.44.2.646-648.2006
8. Lass, A., Szostakowska, B., Korzeniewski, K., & Karanis, P. (2017). The first detection of Toxoplasma gondii DNA in environmental air samples using gelatine filters, real-time PCR and loop-mediated isothermal (LAMP) assays: qualitative and quantitative analysis. *Parasitology*, *144*(13), 1791-1801. doi:10.1017/S0031182017001172
9. Li, Z., Li, T., Yang, L.-T., Deng, C.-Q., Liu, Q.-X., Zhang, Q., Wu, L., Sun, Y., Zou, F.-C., Zhou, X., & Liu, Q.-S. (2026). Bioassay of the infectivity of heat-treated *Toxoplasma gondii* cysts in susceptible C57BL/6J mice. *Food and Waterborne Parasitology*, e Collection 2026 Mar, e00315. https://doi.org/10.1016/j.fawpar 2026.e00315
10. Montoya JG and Liesenfeld O. (2004). Toxoplasmosis. *The Lancet*, 363(9425):1965–1976.
11. Montoya, J. G., & Rosso, F. (2005). Diagnosis and management of toxoplasmosis. *Clinics in perinatology*, *32*(3), 705-726.
12. Pistone, D., Bevivino, G., Dipaola, M.G. *et al.* Current and emerging molecular diagnostic approaches in the detection of human parasites. *Parasitol Res* 125, 50 (2026). https://doi.org/10.1007/s00436-026-08660-y
13. Robert-Gangneux, F., & Dardé, M.-L. (2012, April). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews*, 25(2), 264–296. https://doi.org/10.1128/CMR.05013-11
14. Vilela, V. L. R., & Feitosa, T. F. (2024). Recent Advances in *Toxoplasma gondii* Infection and Toxoplasmosis. *Tropical medicine and infectious disease*, *9*(7), 160. https://doi.org/10.3390/tropicalmed9070160
15. World Health Organization. (2023). *Global tuberculosis report 2023*. Geneva: WHO. https://www.who.int/publications/i/item/9789240083851