

Experimental Study: The Therapeutic Effect of Garlic Plant Extract in Respiratory Cryptosporidiosis

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ABSTRACT

This study aimed to evaluate the therapeutic effect of an aqueous garlic extract against respiratory cryptosporidiosis caused by the parasite *Cryptosporidium parvum*, and to determine whether there were histological or molecular changes after treatment. The experiment was conducted on 60 rats (*Rattus norvegicus*) experimentally infected by inhaling a suspension containing parasitic oocysts. These rats were then treated with the extract, metronidazole, or both. Histological examinations showed a significant improvement in the extract-treated group, represented by a decrease in oocyst density and tissue inflammation. The combined treatment (extract + metronidazole) showed better results, with tissues almost completely returning to normal. Molecularly, the study revealed alterations in the sequence of the target genes and a change in the isolate type, as confirmed by the genotyping of two different isolates in the gene bank. This may be attributed to the effect of the highly concentrated cis-aconitic anhydride compound present in the garlic extract, according to HPLC results. The results indicate the ability of plant extracts to induce genetic changes in the parasite, which calls for extensive studies to understand the mechanism of influence at the level of gene expression and the whole genome.

Keywords- Garlic aqueous extract, Genetic type, Respiratory Cryptosporidiosis.

I. INTRODUCTION

Cryptosporidium spp is a globally widespread parasite and is responsible for deaths resulting from waterborne gastrointestinal [1]. In contrast, the disease is considered a disease associated with poverty and has not received any attention from health personnel according to the World Health Organization report, which is why it was included among the neglected tropical diseases [2].

Cryptosporidium parvum is a pathogenic protozoan parasite belonging to the phylum Apicomplexa. It is an opportunistic parasite that causes diseases common to humans and animals. Accompanied by profuse watery diarrhea in gastrointestinal infections [3]

and unexplained cough in respiratory infections [4], as it multiplies within the epithelial cells lining the gastrointestinal tract, particularly in cases of immunodeficiency and severe infections [5,6] Direct contact with the feces of infected animals or infected individuals, or eating, drinking, or inhaling water contaminated with the parasite's oocysts, in addition to aerosols contaminated with the parasite's oocysts, are among the most prominent methods of transmission, causing both gastrointestinal and respiratory infections [7,8] The danger of infection lies in the lack of treatments officially recognized by the World Health Organization, especially for patients with chronic diseases, immunocompromised individuals, and children under the

age of five. Furthermore, the parasite's Oocyst are resistant to available conventional treatments, and its small size makes it difficult to eradicate in the environment [5], making it an environmentally and medically important parasite.

The *Cryptosporidium parvum* parasite contains important molecular genetic diagnostic markers represented by a number of conserved genes, the most prominent of which is the rRNA S18 gene, which is used to identify the parasite species through molecular diagnosis and classification [9]. Using polymerase chain reaction (PCR), a precise technique for detecting genes, identifying species, and distinguishing isolates and genetic mutations at the genetic level.

Recently, given the limited availability of treatments, attention has turned to medicinal plants, extracting their active compounds, and using them as natural treatments. One such plant is garlic (*Allium sativum*), which contains biologically active compounds known for their antiparasitic, antifungal, and antibacterial properties. One of these compounds is allicin. This compound, produced by crushing garlic cloves, has the ability to affect DNA, enzymes, and the cell membrane, and can even affect the DNA of pathogenic cells [10,11]. This study contributes to the field of applied research directed towards alternative medicine and treatment with natural extracts and knowing the source of their effect on the cellular and molecular structure of pathogens, which is what we tried to investigate through the objective of this research in terms of evaluating the therapeutic effect of the aqueous extract of garlic plant on experimental respiratory infection in laboratory rats that were used as an animal model for the study, and based on molecular analyses and histological examinations of the lung and comparing them with the normal state, the diseased state and treatment with the traditional drug and observing the differences and changes in all recorded indicators.

II. METHODOLOGY

1. Laboratory Animals:

Sixty white rats (*Rattus norvegicus*) of both sexes, weighing 200–250 grams, were obtained from the Experimental Animal Unit at the College of Veterinary Medicine, University of Mosul. Rats were randomly assigned to six groups (10 animals per group), ensuring a balance between males and females.

2. Experimental Design:

A double-blind experimental design was applied, where groups were coded to ensure that the examiners of histological and molecular results were unaware of treatment details. The groups were assigned as follows:

1. Negative Control: Uninfected and received no treatment.
2. Positive Control: Infected and untreated.
3. Garlic Group: Infected and treated with an aqueous garlic extract.

4. Metronidazole Group: Infected and treated with metronidazole.
5. Combined Treatment Group: Infected and treated with a combination of garlic extract and metronidazole.
6. Placebo group: Infected and received saline solution devoid of any active ingredient.

3-Infection:

Infection with *Cryptosporidium* spp. was induced by giving each animal 0.25 ml of oocyst suspension (1×10^6 oocysts/ml) via nasal inhalation for three consecutive days, according to a modified method from [12].

4. Garlic Extract Preparation:

Garlic cloves (*Allium sativum*) were air-dried in the shade, then lipids were removed using petroleum ether for 24 hours. The plant mass was then subjected to ethanolic extraction, followed by water infusion, followed by lyophilization of the aqueous extract to obtain the concentrated extract. The sample was then sent for HPLC testing to determine the highest concentration of the active compound [13].

5. Sample Collection:

At the end of the treatment period (14 days after infection), the animals were anesthetized, and lung tissue samples were taken. These samples were divided into two parts:

- One part was fixed in 10% formalin for histological examination.
- The other part was stored at -80°C for use in molecular analyses.

6. Histological Examination:

Lung samples were processed by routine fixation and sectioning, and the sections were then stained with hematoxylin and eosin (H&E), according to the standard method [14].

7. DNA Extraction:

- a. Genomic DNA was extracted from lung tissue using the Geneaid® [15] Genomic DNA Mini Kit (Tissue), according to the manufacturer's protocol, as follows:
 1. ~25 mg of lung tissue was taken and cut into small pieces using sterile instruments.
 2. The sample was added to a tube containing 200 μL of GT Buffer and 20 μL of Proteinase K, then incubated at 60°C for 1–3 hours.
 3. After cooling, 200 μL of GB Buffer was added and incubated at 70°C for 10 minutes.
 4. 200 μL of absolute ethanol was added, then the mixture was transferred to a DNA spin column and centrifuged.
 5. The column was washed with W1 and Wash Buffer solutions.
 6. The sample was centrifuged to completely dry the column.
 7. 100 μL of Elution Solution was added, left for 3 minutes, and then centrifuged.

8. DNA concentration and purity were measured using a Nano Drop™ spectrophotometer, and the DNA was stored at -20°C until use.

8. Gene Amplification (PCR Amplification):

Polymerase chain reaction (PCR) was performed to detect the presence of *Cryptosporidium parvum* by

amplifying the 18S rRNA gene, using specific primers selected based on previously published studies [16,17]. The reaction was prepared in a final volume of 25 µL using a mixture containing: 12.5 µL of prepared Master Mix (2x), 1 µL of each primer (10 pmol/µL), 2 µL of template DNA, and 8.5 µL of nuclease-free water.

Table (1): Sequences of primers used to amplify the rRNA 18S gene of *Cryptosporidium parvum*

Primer name	Sequence (5'→3')	Target gene	Sequence source
18S-F	5'-AACCTGGTTGATCCTGCCAGTAG-3'	Amplification start	Xiao et al., 1999
18S-R	5'-TGCAGAGCTCGTTTAACTAGC-3'	Amplification end	Xiao et al., 1999

PCR was performed in a thermocycler using the following thermal program:

- Primer activation at 94°C for 5 minutes
- 35 cycles of:
 - 94°C for 30 seconds (denaturation)
 - 58°C for 30 seconds (primer binding)
 - 72°C for 1 minute (extension)
- Final termination at 72°C for 7 minutes

PCR products were used in the next step for cross-validation. Electrophoresis was performed on a 1% agarose gel stained with ethidium bromide (EtBr), with the target bands visualized under UV light.

9. Gene Product Verification:

PCR products were verified using electrophoresis on a 1% agarose gel stained with ethidium bromide (EtBr), and the gel was visualized under UV light.

10. Metronidazole Treatment

A 1 ml dose of the drug was administered orally at a concentration of 1 mg/ml for 5 days. Some rats were treated with the drug alone, while others were treated with a combination of the drug and garlic extract.

III. RESULTS AND DISCUSSION

Histological microscopic examinations using hematoxylin and eosin (H&E) staining revealed severe pathological changes in the lung tissue of the positive control rats, including extensive necrosis, shedding of the bronchiolar lining, and extensive infiltration of inflammatory cells. Oocysts of *Cryptosporidium parvum* were also observed within the alveoli (Figure 1), indicating the success of the experimental intranasal infection model. These changes are consistent with literature reporting the parasite's ability to infect the lungs in severe cases, especially in immunocompromised individuals [8,7]. In contrast, the normal lung tissue of rats, shown in (Figure 2), showed a gradual similarity to that of the group of rats treated with aqueous garlic extract. There was a marked histological improvement, represented by a decrease in inflammatory infiltration and a near-complete return to the normal tissue structure shown in (Figure 3). Meanwhile, the combination treatment group (garlic + metronidazole) showed a better response (Figure 4). Due to its rapid absorption by the intestine and its cytotoxicity after ingestion, and

according to the hypothesis that MTZ indirectly inhibits DNA synthesis, it was used as an antibiotic in the experiment [18], characterized by a clear reorganization of the alveolar structure and a significant reduction in inflammatory markers compared to the other groups. The infected group treated with the traditional drug metronidazole alone showed partial improvement, with moderate inflammation (Figure 5). HPLC results for the garlic plant extract were consistent with those of [19]. These results indicate that cis-Aconitic anhydride, the highest concentration of the aqueous garlic extract, may play a role in accelerating healing by inhibiting parasitic activity and reducing the inflammatory response [11]. Infection was confirmed using Ziehl-Neelsen staining, which revealed the presence of acid-fast oocysts within the lung tissue, supporting the diagnosis of respiratory infection and reinforcing the hypothesis of inhalation transmission, especially in immunocompromised conditions [6].

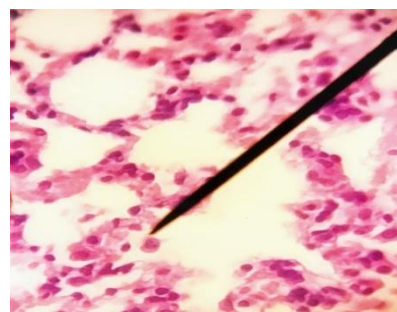


Figure (1): Histological section of the lungs of rats (negative) showed Oocyst of parasite Stained with modified Ziehl-Neelsen stain under 40x.

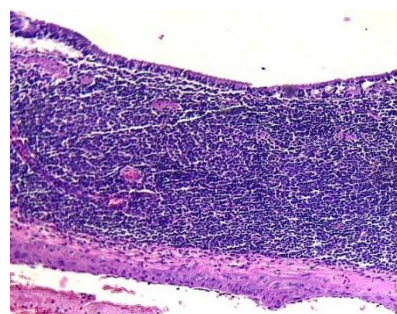
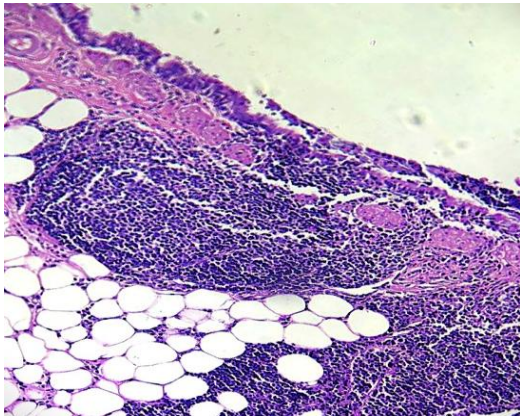
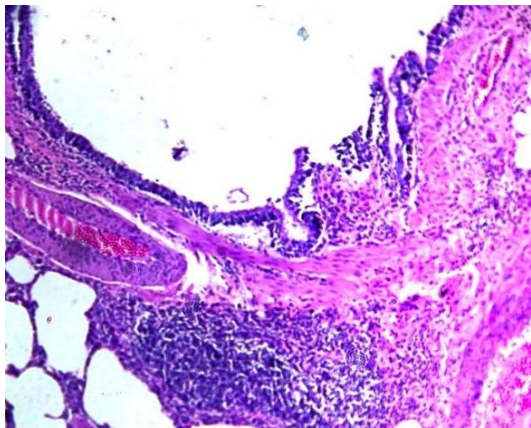


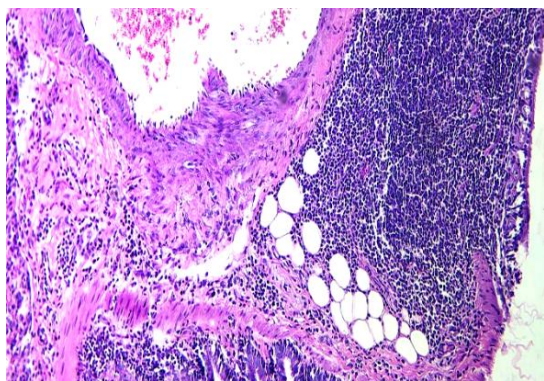
Figure (2): Histological section of the lungs of rats (positive) natural tissue



Figuer (3): Histological section of the lungs of rats treated with aqueous extract of garlic



Figuer (4): Histological section of the lungs of rats treated metronidazole.



Figuer (5): Histological section of the lungs of rats treated with aqueous extract of garlic and metronidazole

At the molecular level, polymerase chain reaction (PCR) showed significant amplification of the 18S rRNA gene with a molecular weight of approximately 600 base pairs in all infected groups, while the negative control group showed no amplification signal (Figure 6), confirming the sensitivity and specificity of the assay [9,16]. Interestingly, the genomic sequencing of isolates from garlic-treated groups revealed significant SNPs compared to untreated isolates, and these changes

were documented by entry in the Gen Bank database (Figures 7 respectively). (LC794437), (LC794438).

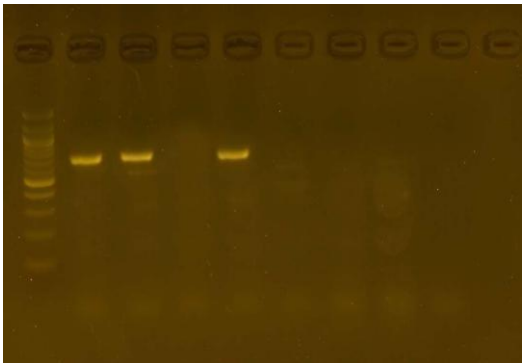


Figure (6): Examination result of PCR 600 bp

LOCUS	LC794437	674 bp	DNA	linear	INV 12-JAN-2024
DEFINITION	Cryptosporidium parvum LF1 gene for 18S rRNA, partial sequence.				
ACCESSION	LC794437				
VERSION	LC794437.1				
KEYWORDS					
SOURCE	Cryptosporidium parvum				
ORGANISM	Cryptosporidium parvum				
REFERENCE	1 (bases 1 to 674)				
AUTHORS	Saloom, L.A. and Alkhashab, F.W.				
TITLE	Direct Submission				
JOURNAL	Submitted (10-JAN-2024) to the DDBJ/EMBL/GenBank databases.				
COMMENT	Contact: Lina Abdulhadi Saloom				
FEATURES	Location/Qualifiers				
BASE COUNT	216 a 38 c 148 g 210 t				
ORIGIN	1 tctgcttgg acggcgaggt attgccttcc cgttgccgtt acggctaac ggccttggg				
	61 ttgtatctc agagagagag cctgagagac gctacacac tctagagag cagagagc				
	121 cgcacatcc ccacccctaa tccagagag tagtcagacg aataacacg acagctctt				
	181 ttgttcttg aattgagtg agttagatg aaacccctt acagatcac attgagggc				
	241 agttcttga ctagagagc aggtatctc agccttaca gcttattat agtttgagc				
	301 agtttaaac cttcagctg gatttctgt aataattat aataattat ttgagata				
	361 ttatataat attacataa tctatcttcc tattttttt ttatattga aattacttt				
	421 ggaataata taattctat agagagaga tacttgatg actctcttc agagatat				
	481 taataattt ttcttctta attgtctta cgtatagat aattgtae agggagagc				
	541 ttggaggtt gttatttca agcgagagc aattattct attgttata gcagacaaa				
	601 tgcacaca ttcttcagg attgtctct tcttcaga acaagata caggttaga				
	661 aacgtcaga taac				
LOCUS	LC794438	387 bp	DNA	linear	INV 12-JAN-2024
DEFINITION	Cryptosporidium parvum LF2 gene for 18S rRNA, partial sequence.				
ACCESSION	LC794438				
VERSION	LC794438.1				
KEYWORDS					
SOURCE	Cryptosporidium parvum				
ORGANISM	Cryptosporidium parvum				
REFERENCE	1 (bases 1 to 387)				
AUTHORS	Saloom, L.A. and Alkhashab, F.W.				
TITLE	Direct Submission				
JOURNAL	Submitted (10-JAN-2024) to the DDBJ/EMBL/GenBank databases.				
COMMENT	Contact: Lina Abdulhadi Saloom				
FEATURES	Location/Qualifiers				
BASE COUNT	134 a 45 c 71 g 137 t				
ORIGIN	1 aattcttca taataattt ccttcttgc agtatata gttataaac aattctatt				
	61 actatttca gttatagaa ttcttcttg agaaattg agttttaa ggaactttt				
	121 gctctgata cttcagatg gaataatat caagatttt tattctttt attgtctta				
	181 agataaaat aattgattat aggcagctt gggcgattt gttattaca gttgaggtt				
	241 aattcttag attgtttaa gaaacagtg gcgagaga tttcgagag attgtttat				
	301 taatcagaa cgaattatg aggtatcag agtatcatg acccttcag tcttaaac				
	361 aactatgcc aactagatg ttgaggt				

Figure (7): Isolation 37 and Isolation 38 of the cryptosporidium parvum parasite registered in the World GenBank

To enhance the clarity of the results, the following table shows a comprehensive comparison between the different experimental groups:

Table (2): Comparison between the experimental groups in terms of histological and molecular examinations

Group	Description	Histological Examination (H&E) Results	PCR (18S rRNA) Results	18S Genome Sequencing Analysis
Negative control	Uninfected and untreated	Normal tissue, free of inflammation	Negative	Not Available
Positive control	Infected and untreated	Severe necrosis and inflammation, evident oocysts	Positive	Standard Sequencing
Garlic group	Infected + treated with garlic extract	Significant histological improvement, decreased inflammation	Positive	Clear Point Changes

To assess quantitative differences between groups, statistical analysis was performed on tissue damage scores using the Kruskal-Wallis test, which showed a significant difference between groups ($H = 12.74$, $p = 0.026$), supporting the hypothesis that the therapeutic extract improved disease outcomes. The chi-square test was also used to analyze genetic changes, which also showed a significant difference between the treated and untreated groups ($\chi^2 = 5.33$, $p = 0.021$), potentially indicating that the garlic extract exerts selective pressure on the parasite, potentially leading to mutations or modifications in conserved genes. Taken together, the results indicate that garlic extract, either alone or in combination with metronidazole, is a promising therapeutic option against respiratory infection with *Cryptosporidium parvum*. Ozlem's findings indicate that *Cryptosporidium* spp should be considered in asthma patients, and molecular testing of these neglected eukaryotic organisms in respiratory tract samples may be useful in the clinical management of the disease, as the presence of parasite oocysts may affect the clinical course of asthma [3].

The study not only demonstrated a significant improvement in damaged lung tissue, but also provided preliminary evidence of a direct effect of plant compounds on the parasite's genetic makeup. This opens the door to using garlic as an alternative or complementary natural remedy, especially in resource-limited settings. However, these results require future studies using whole-genome sequencing and gene expression analysis to determine the precise mechanisms of this molecular effect.

IV. CONCLUSION

The combined results indicate the possibility of using Aqueous garlic extract in the treatment of cryptosporidiosis, either as a supplement to a drug or using different concentrations through laboratory experiments on different animal models. Therefore, extensive experiments should be conducted on the mechanism of action of the active compound on the parasite's egg sacs and an extensive analysis of the genome.

REFERENCES

[1] Hassan, E. M., Örmeci, B., DeRosa, M. C., Dixon, B. R., Sattar, S. A., & Iqbal, A. (2021). A review of *Cryptosporidium* spp. and their detection in water. *Water Science and Technology*, 83(1), 1–25. <https://doi.org/10.2166/wst.2020.515>

[2] Ortega-Pierres, G., Cacciò, S., Fayer, R., Mank, T. G., Smith, H. V., & Thompson, R. C. A. (2009). *Giardia and Cryptosporidium: From molecules to disease*. <https://doi.org/10.1079/9781845933913.0000>

[3] Chen, X. M., Keithly, J. S., Paya, C. V., & LaRusso, N. F. (2002). Cryptosporidiosis. *New England Journal of Medicine*, 346(22), 1723–1731. <https://doi.org/10.1056/NEJMra013170>

[4] Iroh Tam, P. Y., Chisala, M., Nyangulu, W., Thole, H., & Nyirenda, J. (2021). Respiratory cryptosporidiosis in Malawian children with diarrheal disease. *PLoS Neglected Tropical Diseases*, 15(7), e0009643. <https://doi.org/10.1371/journal.pntd.0009643>

[5] Pardy, R. D., Wallbank, B. A., Striepen, B., et al. (2024). Immunity to *Cryptosporidium*: Insights into principles of enteric responses to infection. *Nature Reviews Immunology*, 24, 142–155. <https://doi.org/10.1038/s41577-023-00932-3>

[6] Bagci, O. U., Goksel, O., Gumusburun, R., Mert, U., Bogatekin, G., Toz, S., & Caner, A. (2024). Molecular prevalence of *Pneumocystis jirovecii* and *Cryptosporidium* in patients with asthma. *Diagnostic Microbiology and Infectious Disease*, 108(1). <https://doi.org/10.1016/j.diagmicrobio.2023.116124>

[7] Bouzid, M., Hunter, P. R., Chalmers, R. M., & Tyler, K. M. (2013). *Cryptosporidium* pathogenicity and virulence. *Clinical Microbiology Reviews*, 26. <https://doi.org/10.1128/cmr.00076-12>

[8] O'Connor, R. M., Wanyiri, J. W., Cevallos, A. M., Priest, J. W., & Ward, H. D. (2007). *Cryptosporidium parvum* glycoprotein gp40 localizes to the sporozoite surface by association

- with gp15. *Molecular and Biochemical Parasitology*, 156(1), 80–83. <https://doi.org/10.1016/j.molbiopara.2007.07.010>
- [9] O’Leary, J. K., Blake, L., Corcoran, G. D., Sleator, R. D., & Lucey, B. (2020). Increased diversity and novel subtypes among clinical *Cryptosporidium parvum* and *Cryptosporidium hominis* isolates in Southern Ireland. *Experimental Parasitology*, 218, 107967. <https://doi.org/10.1016/j.exppara.2020.107967>
- [10] Shani, J., Said, O., Saad, B., & Kadan, S. (2021). Antiparasitic and antioxidant properties of *Allium sativum* (garlic) extract: Potential in treating protozoal infections. *Journal of Ethnopharmacology*, 275, 114118. <https://doi.org/10.1016/j.jep.2021.114118>
- [11] Harborne, J. B. (1984). *Phytochemical methods: A guide to modern techniques of plant analysis* (2nd ed.). Springer.
- [12] Zhao, W., Wang, Y., Zhang, C., Liu, W., Liu, A., & Yu, X. (2020). A mouse model of cryptosporidiosis using a natural isolate from humans. *Parasites & Vectors*, 13(1), 1–10. <https://doi.org/10.1186/s13071-020-04407-z>
- [13] Al-Khashab, F. M., & L. A., S. N. (2022). Evaluation of the therapeutic efficacy of alcoholic extract of tannins (before and after acidosis) on laboratory mice experimentally infected with the parasite *Cryptosporidium parvum*. *Journal for Research in Applied Sciences and Biotechnology*, 1(3), 138–145. <https://doi.org/10.55544/jrasb.1.3.19>
- [14] Bancroft, J. D., & Gamble, M. (2008). *Theory and practice of histological techniques* (6th ed.). Churchill Livingstone Elsevier.
- [15] Geneaid Biotech Ltd. (2023). *Genomic DNA Mini Kit (Tissue) – Instruction manual*. <https://geneaid.com/data/files/1605685391109197921.pdf>
- [16] Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A. A., Montali, R. J., Fayer, R., & Lal, A. A. (1999). Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. *Applied and Environmental Microbiology*, 65(4), 1578–1583. <https://doi.org/10.1128/AEM.65.4.1578-1583.1999>
- [17] Pedraza-Díaz, S., Amar, C., Nichols, G. L., & McLauchlin, J. (2001). Nested polymerase chain reaction for amplification of the *Cryptosporidium* oocyst wall protein gene. *Emerging Infectious Diseases*, 7(1), 49–56. <https://doi.org/10.3201/eid0701.700049>
- [18] Dingsdag, S. A., & Hunter, N. (2018). Metronidazole: An update on metabolism, structure-cytotoxicity and resistance mechanisms. *Journal of Antimicrobial Chemotherapy*, 73(2), 265–279. <https://doi.org/10.1093/jac/dkx351>
- [19] Aziz, A. S., Tawfeeq, F. K., & Ahmed, M. S. (2024). Therapeutic role of vitamin D3, K2, garlic extract, calcium and spirulina algae powder on induced hyperlipidemia in rats. *Egyptian Journal of Veterinary Sciences*, 55(3), 651–659. <https://doi.org/10.21608/ejvs.2023.242151.1640>