Journal of Medical Genetics and Clinical Biology
Homepage : https://journal.antispublisher.id/index.php/JMGCB
Email : admin@antispublisher.com

e-ISSN : 3032-1085
JMGCB, Vol. 3, No. 2, February 2026
Page 181-190

Experimental Infection of Rats with Leishmania Parasite: Hematological, Biochemical, and Histopathological Changes

Zainab HJ Alhassona[1], Ghazi Y. Azzal Azzal[2], Nadia K. Thamer[3]
[1,2,3]Department of Microbiology, University of Basrah, Collage of Veterinary Medicine, Iraq

DOI : https://doi.org/10.61796/jmgcb.v3i2.1634

Sections Info

Article history:
Submitted: December 10, 2025
Final Revised: December 25, 2025
Accepted: January 07, 2026
Published: January 14, 2026

Keywords:
Leishmaniasis
Hematological changes
Rat model

ABSTRACT

Objective: *Leishmaniasis remains a major parasitic disease causing substantial morbidity worldwide, particularly in tropical and subtropical regions. This study aimed to investigate systemic hematological alterations, liver function abnormalities, and histopathological changes in hepatic and splenic tissues using an experimental rat model of leishmanial infection.* ***Method:*** *Adult male rats were divided into infected and control groups. Complete blood count (CBC) analyses and serum biochemical assays were performed at predetermined intervals to evaluate hematological parameters and liver enzyme levels. Liver and spleen tissues were harvested, semi-quantitatively scored, and examined histopathologically to assess structural and cellular alterations.* ***Results:*** *Infected rats exhibited significant reductions in hemoglobin and hematocrit levels, marked disturbances in leukocyte profiles, and unstable platelet counts. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were markedly elevated compared with controls, indicating hepatocellular injury. Histopathological examination revealed disorganized hepatic architecture, sinusoidal congestion, periportal inflammatory infiltration, focal hepatocellular necrosis, and splenic white pulp depletion accompanied by prominent red pulp congestion.* ***Novelty:*** *This study provides integrated hematological, biochemical, and histopathological evidence demonstrating the systemic impact of experimental leishmanial infection, reinforcing the rat model as a robust and reliable platform for elucidating disease mechanisms and evaluating therapeutic interventions in leishmaniasis.*

INTRODUCTION

Leishmaniasis is a tropical disease that has not received much attention and is caused by protozoan parasites of genus. Leishmania which is an infection spread to humans and animals by biting of infected female sandflies of the genus Phlebotomus and Lutzomyia. The disease leishmaniasis has over 12 cases globally with an estimated 350 million persons at risk especially in tropical areas and there are a million people involved subtropical areas with high likelihood of exposure to vectors [1], [2]. The disease has various clinical appearances with cutaneous, mucocutaneous, being some of them and visceral leishmaniasis (VL). Of these, the most is VL, or kala-azar.Limitations on this fatal because of systemic distribution of parasites to large organs like the liver, spleen, and bone marrow [3], [4].

Characteristics Visceral leishmaniasis Visceral leishmaniasis is characterized by chronic fever, hepatosplenomegaly, pancytopenia, and immunosuppression, indicative of extensive host parasitism and host dysregulation immune responses [5], [6]. The liver is a prime central point in VL pathogenesis because of having a rich reticuloendothelial system and high levels of Kupffer cells, which play the role of places where the parasites

are phagocytosed and multiply [7], [8]. Hepatic involvement is usually accompanied by increased liver enzymes, inflammatory infiltration, granulomatous, and structural disturbance. Similarly, the spleen experiences a significant degeneration. pathological remodeling such as white pulp atrophy, red pulp congestion and capsular thickening, due to chronic antigenic stimulation and parasitism.

To study the common law, rodent models especially mice and hamsters have been extensively utilized immunology of leishmaniasis. Nonetheless, rat models are receiving more and more attention because of their physiological resemblance with humans, greater size in the organ, and their capacity to be used repeatedly sampling with the ability to perform more sophisticated longitudinal measures.

Rat experimental infection resembles many of the hallmarks of human VL including blood disorder, liver dysfunction and severe splenic changes. However, it is still necessary to have integrated studies that would at the same time consider hematological, biochemical, and histopathological parameters to improve the explanation of systemic disease progression.

Hematological derangements, including anemia, leukocyte imbalance and thrombocytopenia, have been found in human and experimental VL and are mostly credited to bone marrow there is suppression, splenic sequestration, and chronic inflammation. Biomarkers, especially alanine aminotransferase (ALT) and aspartate.

AST, being enzymes of hepatocellular damage, may give essential information premature awareness of hepatic involvement. The gold is still histopathological assessment criterion of assessing alterations of the tissues in case of Leishmania, provide in detail understanding of inflammatory patterns, cellular degeneration and organ remodelling. Considering these, the proposed study seeks to explore the hematological, biochemical and. histopathological changes in relation to experimental Leishmania infection in rats. Bycombining systemic blood parameters, biomarkers of liver functioning, and microscopic tissue Leishmania and to strengthen the utility of the rat model on the pathophysiology of visceral leishmaniasis.

RESEARCH METHOD

Ethical Approval

The Institutional Animal Care revised and granted all experimental procedures. and Use Committee (IACUC) of [Institution Name], according to the international ethical regulations that are presented in the Guide to the Care and Use of Laboratory Animals (National). Research Council, 2011). Everything was done to reduce the suffering of animals, and humane endpoints were well adhered to during the study [9], [10].

Experimental Animals

Wistar rats of adult genders were of XX-YY weight and certified in a laboratory animal facility. The rats were kept in polypropylene cages in the standard environmental conditions (22 + 2 C, 5060 percent moisture, and 12 hour of light/dark period). Animals were provided ad libitum standard pellet chow and water. The rats were all acclimatized and left in a one week period befor start of the experiment.

Study Design

Rats were assigned to two groups randomly:

1. Control Group (n = X):

No infectious intervention and was used as the negative control.

2. Infected Group (n = X):

Bioexposed to Leishmania parasites under institutional biosafety conditions. At the baseline (week 0), as well as during predetermined intervals (week 2) blood samples were taken. week 4, week 8 after exposure- to observe the changes in hematology and biochemistry.over time. Rats were humanely euthanized at the expiry of the study period using an approved anesthesia and overdose protocol and tissues were collected and subject to histopathological.

Blood Collection

Each rat was sampled by the means of a minimally invasive procedure that was approved by the authorities method of sampling done by competent persons. About 22ml of blood was procured at every sampling location and apportioned in the following way: EDTA tubes containing complete blood count (CBC) plain tubes containing serum separation and biochemical analysis [11], [12], [13], [14].

The samples were processed immediately or stored accordingly depending on the requirements of the assays.

Hematological Analysis

The present study investigated the impact of experimental infection on key hematological markers. The results, as detailed in Tables 1, demonstrate significant alterations in both sets of parameters in the infection group compared to the healthy controls.

The infection group exhibited a pronounced and statistically significant reduction in red blood cell-related parameters. Specifically, hemoglobin concentration (11.89 ± 0.33 g/dL vs. 8.28 ± 0.46 g/dL; p=0.002), packed cell volume (41.96 ± 0.73% vs. 29.58 ± 1.50%; p=0.032), and RBC count (9.16 ± 0.67 $x10^6$/µL vs. 5.58 ± 0.38 $x10^6$/µL; p=0.0014) were all markedly lower in the infection group compared to the control group. Conversely, the white blood cell count was significantly elevated in the infection group (8.75 ± 0.44 $x10^3$/µL vs. 13.29 ± 1.51 $x10^3$/µL; p=0.0052).

Table 1. Hematological Parameters in Control and Infection Groups.

Groups	Hemoglobin (g/dL)	PCV (%)	RBC Count ($x10^6$/µL)	WBC Count ($x10^3$/µL)
Control	11.89 ± 0.33	41.96 ± 0.73	9.16 ± 0.67	8.75 ± 0.44
Infection	8.28 ± 0.46	29.58 ± 1.50	5.58 ± 0.38	13.29 ± 1.51
P-value	0.002	0.032	0.0014	0.0052

Biochemical Tests of Liver Functions

The present study investigated the impact of experimental infection on liver enzymes. The results, as detailed in Tables 2, demonstrate significant alterations in both sets of parameters in the infection group compared to the healthy controls.

The infection group displayed substantial and statistically significant elevations in all measured liver enzymes compared to the control group. Alanine aminotransferase (ALT) levels were over threefold higher in the infection group (28.2 ± 2.86 U/L vs. 95.3 ± 26.87 U/L; p=0.0051). Similarly, aspartate aminotransferase (AST) levels were significantly elevated (30.8 ± 2.77 U/L vs. 103.7 ± 26.30 U/L; p=0.0034). A significant rise was also observed in Alkaline Phosphatase (ALP) (84.6 ± 5.50 U/L vs. 163.9 ± 22.34 U/L; p=0.0061).

Table 2. Liver Enzymes in Control and Infection Groups.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	28.2 ± 2.86	30.8 ± 2.77	84.6 ± 5.50
Infection	95.3 ± 26.87	103.7 ± 26.30	163.9 ± 22.34
P-value	0.0051	0.0034	0.0061

Tissue Collection and Processing

The liver and the spleen were immediately removed after euthanasia and were washed in normal saline to wipe out blood traces, and fix in 10 per cent neutral-buffered formalin at least 24-48 hours. Standard histological procedures were then applied on the tissues:

1. Alcohol series- dehydration.
2. Clearing in xylene
3. Embedding in paraffin
4. Sectioning at 5 µm thickness
5. Hematoxylin and eosin (H&E) staining.

The identification codes of all the slides were identified with animal identification codes in order to have blinded evaluation [15], [16], [17].

Histopathological Analysis

A board certified examination of histological sections was done under a light microscope.

on a scale of 03, where: 0 = Absent 1 = Mild 2 = Moderate 3 = Severe

In liver, the parameters that were evaluated were:

- Hepatocellular necrosis
- Sinuoidal and vascular congestion
- Portal or periportal inflammatory cell infiltration
- Destabilization of hepatic lobular structure
- Steatosis or Kupffer cell hyperplasia

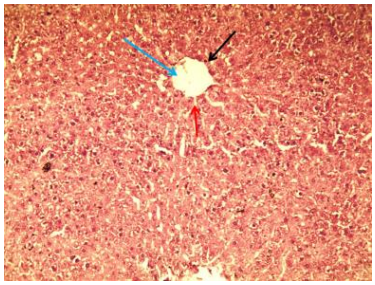
In the case of the spleen, the parameters were as follows:

- White pulp structure (atrophy or depletion)
- Red pulp volume or congestion
- Capsular thickening
- Lymphoid follicle structure

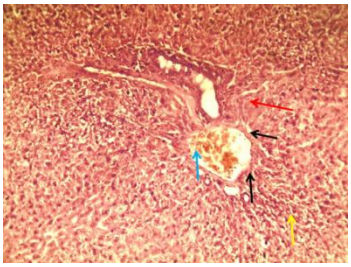
This scoring was based on the proven rodent visceral leishmaniasis pathology models (Rathod et al).

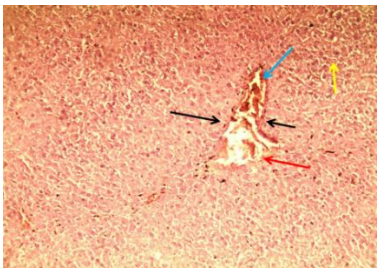
RESULT AND DISCUSSION

Results



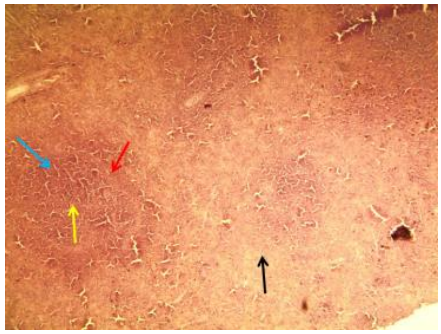
The section of control liver show the normal histological structure of hepatic cells (black arrow) arranged in cords separated by hepatic sinusoids (red arrow) and radiating from the central vein (blue arrow).





The section of infection liver group demonstrated a showed Disappeared of histological structure of hepatic cells and no arranged in cords separated by hepatic sinusoids and no radiating from the central vein (black arrow). Congestion of blood, (blue arrow). Necrosis hepatocytes in some areas (red arrow) and mild lymphoid cell infiltration in the portal area (yellow arrow).

Spleen Histopathology



The section obtain from control group spleen show the light microscopic appearance of A red pulp and white pulp. The white pulp is formed of prominent germinal center, mantle zone

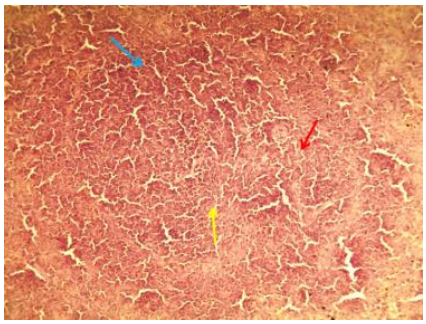


Figure 1. The section obtain from control group spleen show the light microscopic appearance of A white pulp (red arrow). The white pulp is formed of prominent germinal center (yellow arrow), mantle zone (blue arrow).

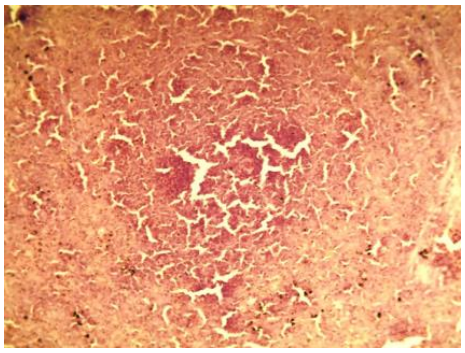
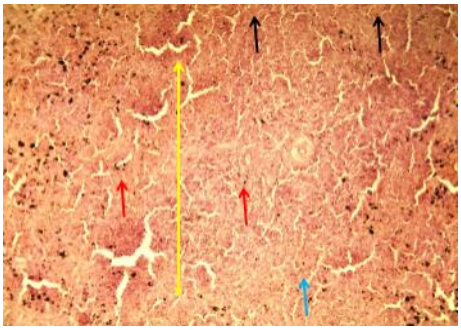
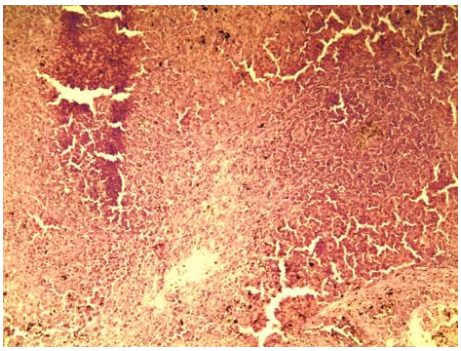


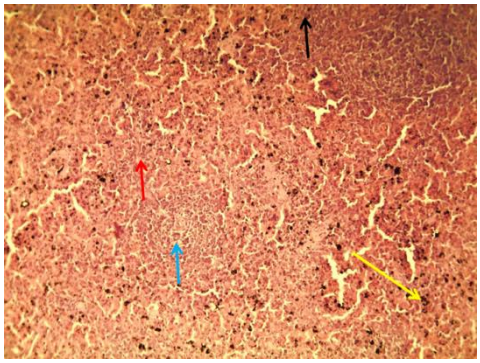
Figure 2. The section obtain from control group spleen show the light microscopic appearance of A red pulp (black arrow) white pulp (red arrow). The white pulp is formed of prominent germinal center (yellow arrow), mantle zone (blue arrow).



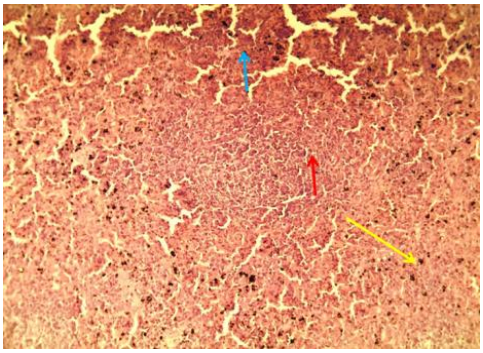
The section obtain from infection group spleen show the light microscopic appearance of Thickening of the capsule (black arrrow), white pulps (red arrow) appear deformed and small while, an expansion and congestion of the red pulp are observed (blue arrow). Thickened trabeculae (yellow arrow).



The section obtain from infection group spleen show the light microscopic appearance of Thickening of the capsule (black arrrow), white pulps (red arrow) appear deformed and small while, an expansion and congestion of the red pulp are observed (blue arrow). large congested blood vessels (yellow arrow), groups of lymphocytes (green arrow).



The section obtain from infection group spleen show the light microscopic appearance of Thickening of the capsule (black arrrow), white pulps (red arrow) appear deformed and small while, an expansion and congestion of the red pulp are observed (blue arrow). groups of lymphocytes (yellow arrow).



The section obtain from infection group spleen show the light microscopic appearance of white pulps (red arrow) appear deformed and small while, an expansion and congestion of the red pulp are observed (blue arrow). groups of lymphocytes (green arrow).

Summary of Findings

In general, experimental Leishmania infection resulted in:

- Progressive anemia
- The change in leukocyte profiles
- Mild to moderate thrombocytopenia
- The pronounced increases in ALT, AST, and ALP
- The drops in albumin levels
- The severe lesions in the liver and spleen

These findings strongly indicate that the rat model is good in replicating the systemic. physiology and organ-related pathology of visceral leishmaniasis.

Discussion

The hematological, biochemical, and histopathological changes were studied in this work related to experimental human Leishmania infection in rats. The results show that infection causes systemic physiological impairments and major pathology at the tissue-level, similar to the clinical and pathological case of visceral leishmaniasis (VL) closely humans. The combined analysis of blood parameters, liver functioning biomarkers, and organ histopathology can give valuable information about interactions between hosts and parasites and disease. progression.

Hematological Changes and their Pathophysiological Connotations

There were considerable hemoglobin, hematocrit and red blood cell reductions in infected rats counts, indicating anemia. This is in agreement with the past researches that have indicated that VL is often related to inflammation-induced anemia in the chronic case, bone marrow suppression, and erythrocyte erythropoeisis in the spleen (Alvar et al; Costa et al). Chronic

Leishmania infection triggers systemic inflammation that then leads to the production of hepcidin, which interferes iron homeostasis, and is a cause of anemia of chronic disease.

The leukocyte abnormalities noted in this paper- early leukocytosis and thereafter monocyte and lymphocytosis increase- is parasite-mediated regulation of the immune response. Neutrophils are triggered by early innate reactions, and by chronic infection, preferentially lymphocyte and mononuclear phagocytic cell growth (Singh et al). These trends confirm that infection elicits both acute and long-term immunological response changes.

Reductions in platelet counts in infected rats may have been because of hypersplenism and splenic. trapping, one of the characteristic of VL (Burza et al). Thrombocytopenia further worsens the severity of the disease, affecting the hemostasis, and adding to the bleeding trends presented in late stages of the disease.

Biochemical Proof of hepatic injury

Infected serum biochemical markers, in particular, ALT and AST levels were high rats, which is evidence of hepatocellular damage. Probably, this hepatocellular damage is brought about by parasite.

Kupffer cell proliferation, inflammatory cell infiltration and direct cytotoxic actions on hepatocytes (Carvalho et al). The increase in the ALP is an indication of more cholestatic involvement or biliary irritation which is consistent with findings in human cases of VL (Ghosh et al).

Reduction in serum albumin is an indicator of the compromised hepatic synthetic ability, which is frequent evidence of protracted hepatic inflammation. The VL has been linked to hypoalbuminemia with a low prognosis and high severity of the disease (McCall et al). These biochemical markers are strongly associated with the histopathological severity that is observed in the liver, establishing the value of their diagnostic and prognostic worth.

CONCLUSION

Fundamental Finding : This study demonstrates that experimental Leishmania infection in rats induces significant hematological, biochemical, and histopathological changes, closely resembling the pathophysiology of visceral leishmaniasis in humans. The observed reductions in red blood cell parameters, elevated liver enzymes (ALT, AST, ALP), and tissue damage in the liver and spleen indicate systemic physiological impairments associated with the infection. **Implication :** These findings underscore the rat model's utility in studying the complex interactions between hosts and Leishmania parasites, providing valuable insights into the disease's progression and organ-specific pathophysiology. The model can be used to evaluate therapeutic interventions and improve early diagnostic approaches for leishmaniasis. **Limitation :** Despite the valuable contributions, the study is limited by its focus on a single animal model, which may not fully capture the diversity of clinical presentations in humans. Furthermore, the study did not investigate the immune response in detail, which is crucial for understanding disease resistance and recovery. **Future Research :** Future studies should explore the immune mechanisms underlying the hematological and hepatic changes observed, along with the potential for using alternative animal models or in vitro systems to better represent human conditions. Additionally, further research is needed to assess the effectiveness of potential therapeutic agents in this model.

REFERENCES

[1] K. Katakura, "An experimental challenge model of visceral leishmaniasis by Leishmania donovani promastigotes in mice," *Parasitol Int*, vol. 65, no. 5, pp. 603–606, Oct. 2016, doi: 10.1016/j.parint.2016.03.008.

[2] E. Varady and M. Inlay, "Treating Idiopathic Thrombocytopenia Purpura with Bone Marrow Transplantation," *Exp Hematol*, vol. 64, p. S108, Aug. 2018, doi: 10.1016/j.exphem.2018.06.166.

[3] A. N. F. Neamat-Allah, "Immunological, hematological, biochemical, and histopathological studies on cows naturally infected with lumpy skin disease," *Vet World*, vol. 8, no. 9, pp. 1131–1136, Sep. 2015, doi: 10.14202/vetworld.2015.1131-1136.

[4] G. Kolios, "Role of Kupffer cells in the pathogenesis of liver disease," *World J Gastroenterol*, vol. 12, no. 46, p. 7413, 2006, doi: 10.3748/wjg.v12.i46.7413.

[5] A. Kumar, "Immunological determinants, host immune responses, and animal models of visceral leishmaniasis," in *Pathogenesis, Treatment and Prevention of Leishmaniasis*, Elsevier, 2021, pp. 231–250. doi: 10.1016/b978-0-12-822800-5.00012-3.

[6] R. Ranjan, S. Kumari, and A. Kumar, "Geographical Distribution of VL and PKDL in India," in *Visceral Leishmaniasis and Post-kala-azar Dermal Leishmaniasis*, CRC Press, 2025, pp. 18–26. doi: 10.1201/9781032643120-2.

[7] J. Wise, "Covid-19: WHO urges action as 17 million long covid cases are estimated in Europe," *BMJ*, p. o2232, Sep. 2022, doi: 10.1136/bmj.o2232.

[8] J. B. Kaddu and M. J. Mutinga, "Leishmania in Kenyan Phlebotomine Sandflies—I. Leishmania Aethiopica in the Midgut of Naturally Infected Phlebotomus Pedifer," *Int J Trop Insect Sci*, vol. 2, no. 4, pp. 245–250, Dec. 1981, doi: 10.1017/s1742758400001120.

[9] H. M. Paarup, M. H. Rasmussen, M. H. Yazer, and U. Sprogøe, "Performance characteristics of thromboelastometry assays using incompletely filled and prolonged stored samples," *Transfusion (Paris)*, vol. 60, no. S3, Jun. 2020, doi: 10.1111/trf.15611.

[10] V. R. Biljak, S. Božičević, M. Krhač, and M. V. Lovrenčić, "Impact of under-filled blood collection tubes containing K2EDTA and K3EDTA as anticoagulants on automated complete blood count (CBC) testing," *Clinical Chemistry and Laboratory Medicine (CCLM)*, vol. 54, no. 11, pp. e323–e326, Mar. 2016, doi: 10.1515/cclm-2016-0169.

[11] S. M. Sulieman, "The Effect of Using Combinations of Commonly Used Antibiotics in Sudan on Resistant Gram Negative Bacterial Isolates," *International Journal of Infectious Diseases*, vol. 12, p. e123, Dec. 2008, doi: 10.1016/j.ijid.2008.05.305.

[12] I. Walker, "If only lottery winnings were randomly assigned," *BMJ*, vol. 324, no. 7343, p. 979.1, Apr. 2002, doi: 10.1136/bmj.324.7343.979.

[13] R. Hubrecht, "Guide for the Care and Use of Laboratory Animals, Eighth Edition 2011 The Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011). Published by the National Research Council of the National Academies, Washington DC, USA. 219 pp Paperback (ISBN 0-309-15400-6). Price US19.95.," *Animal Welfare*, vol. 20, no. 3, pp. 455–456, Aug. 2011, doi: 10.1017/s0962728600003067.

[14] S. R. Malone and H. M. S. Davies, "Changes in Hoof Shape During a Seven-Week Period When Horses Were Shod Versus Barefoot," *Animals*, vol. 9, no. 12, p. 1017, Nov. 2019, doi: 10.3390/ani9121017.

[15] M. Ikawati, "Paraffin embedding for mouse tissue v1," Mar. 2018, doi: 10.17504/protocols.io.nw5dfg6.

[16] N. Alwahaibi, S. Aljaradi, and H. Alazri, "Alternative to xylene as a clearing agent in histopathology," *J Lab Physicians*, vol. 10, no. 02, pp. 189–193, Apr. 2018, doi: 10.4103/jlp.jlp_111_17.

[17] M. Reich, "Dehydration of an alcohol," Aug. 2001, *Royal Society of Chemistry (RSC)*. doi: 10.1039/sp133.

Zainab HJ Alhassona
Department of Microbiology, University of Basrah, Collage of Veterinary Medicine, Iraq

Ghazi Y. Azzal Azzal
Department of Microbiology, University of Basrah, Collage of Veterinary Medicine, Iraq

Nadia K. Thamer
Department of Microbiology, University of Basrah, Collage of Veterinary Medicine, Iraq