

Time-dependent renal histopathological alterations in male albino rats experimentally infected with *Leishmania donovani*

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Abstract

Visceral leishmaniasis is primarily associated with the mononuclear phagocyte system, yet renal involvement may occur through systemic inflammation, immune-complex deposition, hemodynamic disturbance, and direct or indirect tubular injury. This study examined the time-dependent renal histological response to experimental *Leishmania donovani* infection in adult male albino rats. Twenty rats were divided into four groups (n = 5): two time-matched controls receiving intraperitoneal distilled water for 30 or 60 days and two groups inoculated intraperitoneally with 15×10^6 parasites and examined after 30 or 60 days. Kidneys were fixed, paraffin embedded, sectioned at 5 μ m, and stained with hematoxylin and eosin. Serum total protein, albumin, and globulin were analyzed as supportive systemic biochemical variables. At 30 days, infected rats showed focal glomerular atrophy and hyaline casts within renal tubules. At 60 days, glomerular congestion and tubular degeneration were observed. Relative kidney weight did not differ significantly from the corresponding controls, and serum total protein, albumin, and globulin showed no statistically significant differences. The coexistence of descriptive renal lesions with non-significant protein-profile changes indicates that structural renal abnormalities may be detectable before or without marked alteration in the measured serum proteins. The 30- and 60-day patterns were not identical, suggesting temporal evolution from focal glomerular and cast-related changes toward vascular congestion and tubular degeneration. These findings should be interpreted cautiously because only five animals were included per group, renal function markers such as creatinine, urea, urinalysis, and proteinuria were not measured, and histopathology was not quantified. The results support further work integrating renal histology with functional biomarkers, urinary protein assessment, immunopathology, and parasite localization.

Keywords: Visceral leishmaniasis, renal histopathology, glomerular atrophy, tubular degeneration, hyaline casts, albino rats

Introduction

Visceral leishmaniasis is a systemic protozoal disease caused mainly by members of the *Leishmania donovani* complex. After inoculation by an infected sand fly, parasites establish an intracellular niche in macrophages and disseminate to organs rich in mononuclear phagocytes. The liver, spleen, and bone marrow are the classical target organs, but extra-reticuloendothelial involvement is increasingly recognized. Renal abnormalities have been documented in human visceral leishmaniasis and are particularly well characterized in canine leishmaniosis, where glomerular disease and proteinuria can contribute substantially to morbidity (da Silva Junior *et al.*, 2014; Solano-Gallego *et al.*, 2011)^[22, 26].

The kidney may be affected through several non-exclusive mechanisms. Circulating immune complexes may become deposited in glomerular capillary walls or mesangial regions, activating complement and inflammatory pathways. Systemic cytokine release may alter renal perfusion and endothelial function. Tubular epithelial cells may subsequently develop degenerative changes due to ischemia, filtered inflammatory mediators, protein overload, oxidative stress, or altered microcirculation. These pathways are biologically plausible, but conventional hematoxylin-and-eosin sections alone cannot determine which mechanism is dominant.

The present experiment offers a focused opportunity to compare renal morphology at two post-infection intervals

while preserving the distinction between observed lesions and mechanistic interpretation. The 30-day kidney displayed focal glomerular atrophy and tubular hyaline casts, whereas the 60-day kidney showed glomerular congestion and tubular degeneration. These observations indicate that renal involvement was heterogeneous across time. Importantly, kidney weight and serum protein variables were not significantly changed. Therefore, the study does not support a claim of overt renal failure; instead, it documents descriptive tissue alterations compatible with subclinical or evolving renal injury.

The relevance of this distinction is methodological and clinical. Organ weight is a coarse endpoint and can remain unchanged despite microscopic injury. Likewise, serum total protein, albumin, and globulin are influenced by hepatic synthesis, inflammation, nutritional status, vascular permeability, and renal loss. Their stability cannot exclude focal glomerular or tubular damage. A kidney-specific analysis is therefore needed to interpret the histological findings on their own terms and to define what can and cannot be concluded from this experimental design.

Research Problem

Renal involvement in visceral leishmaniasis is frequently overshadowed by hepatic and splenic disease. In experimental studies, renal observations are often reported as secondary findings without a time-specific analysis or without separating direct observations from inferred

immunopathological mechanisms. The central problem addressed here is whether a single intraperitoneal inoculum of 15×10^6 *Leishmania donovani* promastigotes produces recognizable renal histological changes after 30 and 60 days in male albino rats, even when relative kidney weight and measured serum protein variables remain statistically unchanged.

Aim and Objectives

To characterize kidney-specific histological alterations after 30 and 60 days of *Leishmania donovani* infection.

To compare infected kidneys with time-matched control kidneys using descriptive H&E histology.

To relate renal morphology cautiously to relative kidney weight and serum total protein, albumin, and globulin.

To identify plausible mechanisms and define the evidentiary limits of the study.

Materials and Methods

Twenty adult male albino rats (*Rattus* spp.), 2–3 months of age and weighing approximately 224–294 g, were used. Animals were obtained from the animal experimental facility of the College of Science, University of Babylon. They were maintained under standard laboratory conditions and provided commercial dry feed and water. Following acclimatization, animals were allocated into four groups of five rats each. Group 1 received intraperitoneal distilled water and was evaluated at 30 days. Group 2 was inoculated intraperitoneally on day 0 with 15×10^6 *Leishmania donovani* promastigotes and evaluated at 30 days. Group 3 received intraperitoneal distilled water and was evaluated at 60 days. Group 4 was inoculated with the same parasite dose and evaluated at 60 days.

The parasite was propagated in Novy–MacNeal–Nicolle medium and subsequently maintained in RPMI 1640 supplemented with fetal bovine serum, HEPES, and gentamicin. Parasite viability and the promastigote morphology were checked microscopically before inoculation. At the designated endpoints, animals were anesthetized and dissected. Organs were removed for gross weighing and histological examination. The study protocol was approved by the institutional ethics board of the College of Science, University of Babylon (approval Z240802, dated 28 August 2024).

Experimental Design

The design was a controlled, four-group, two-time-point animal experiment. Each infected group had a separate time-matched control, limiting confounding by age and duration of housing. The exposure was a single intraperitoneal parasite inoculation at day 0. The principal renal endpoint was descriptive histology. Relative kidney weight and serum protein measurements were treated as supportive endpoints, not as direct measures of glomerular filtration or tubular function.

Histological Tissue Preparation

Immediately after dissection, tissue specimens were rinsed with normal saline and fixed for 24 h in formalin. Samples were processed through graded ethanol concentrations, cleared in xylene, infiltrated and embedded in paraffin wax. Paraffin blocks were trimmed and sectioned at approximately 5 μ m using a rotary microtome. Sections were floated on a warm water bath, mounted on albumin-coated glass slides, deparaffinized, rehydrated, and stained with Harris hematoxylin and 1% eosin. Following

dehydration and clearing, sections were mounted with DPX and examined by compound light microscopy. Photomicrographs were obtained using a camera attached to the microscope. Histological interpretation was descriptive and comparative; no lesion-scoring system, morphometric analysis, stereology, immunohistochemistry, or quantitative parasite-burden assessment was reported.

Statistical Analysis

Continuous biochemical and weight data were analyzed with SPSS version 23.0 and summarized as mean $\pm$ standard deviation. One-way analysis of variance was used, followed by Duncan's multiple-range procedure for mean separation. Statistical significance was set at $P \leq 0.05$. Histological observations were evaluated descriptively by comparing infected groups with their time-matched controls. Because the histological component was not quantified, inferential statistics were not applied to lesion frequency or severity.

Results

The renal parenchyma of control animals at both time points retained the expected general organization of cortical renal corpuscles and tubular profiles. In the 30-day infected group, kidney sections showed focal glomerular atrophy. The lesion was localized rather than described as diffuse. Hyaline casts were also observed within renal tubules, indicating intraluminal accumulation of eosinophilic material. The study did not report cast counts, segment-specific localization, or a semiquantitative severity grade.

At 60 days, the reported pattern differed. Glomerular congestion was present, accompanied by tubular degeneration. The available description supports vascular engorgement within glomerular capillary tufts and degenerative alteration of tubular epithelium, but it does not establish the precise tubular segment, the presence of necrosis, or the reversibility of the lesion. No immunohistochemical or ultrastructural confirmation was performed.

Relative kidney weight showed no significant difference between infected and control groups at either time point. Serum total protein also showed no significant change: 5.58 ± 0.61 g/dL at 30 days and 4.603 ± 0.624 g/dL at 60 days in infected rats, compared with 5.50 ± 0.75 and 5.043 ± 1.24 g/dL in the respective controls. Albumin values were 3.44 ± 0.22 and 2.50 ± 0.80 g/dL in infected animals compared with 3.50 ± 0.16 and 2.77 ± 0.37 g/dL in controls, with no significant differences. Globulin was likewise reported as non-significant. Because a probable transcription inconsistency is present in the thesis for the 60-day globulin value, that numerical value is not repeated here; the inferential conclusion reported in the source was retained.

Taken together, the data show microscopic renal abnormalities in the absence of a significant change in kidney weight or the measured serum protein profile. No claims can be made regarding serum creatinine, blood urea nitrogen, electrolyte handling, urinary protein loss, or glomerular filtration because these variables were not measured.

Kidney

As shown in Figure (4-10), the histological section of kidney in male rats treated with parasite for 30 days showed focal glomerular atrophy and revealed hyaline cast in the renal tubule as compared with control group. While the histological section of kidney in male rats treated with parasite for 60 days showed normal architecture of tissue as compared with control group (Fig.1,2).

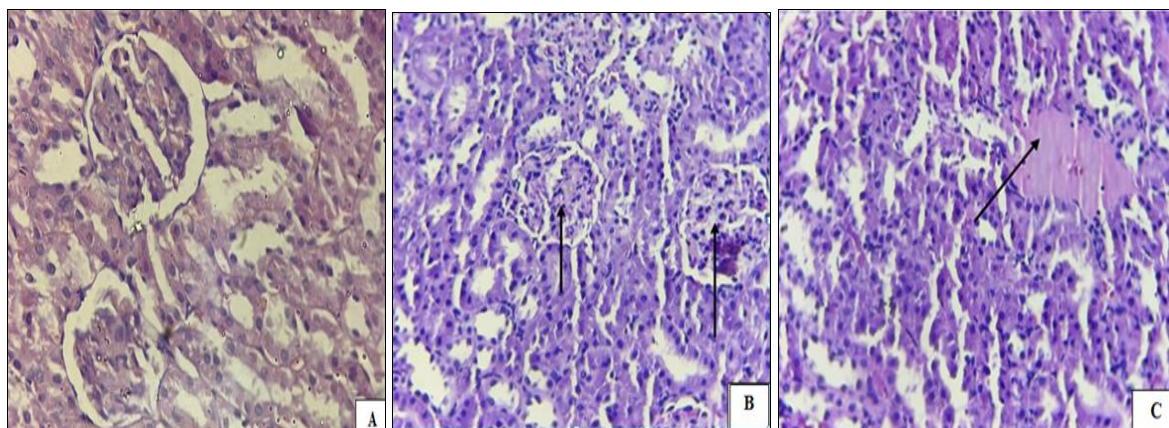


Fig 1: Transvers section of male albino rat's kidney. A: Control group, B, C: Treated with *L. donovani* for 30 days demonstrate focal glomerular atrophy and hyaline cast in the renal tubule respectively .H&E–stain. (X40)

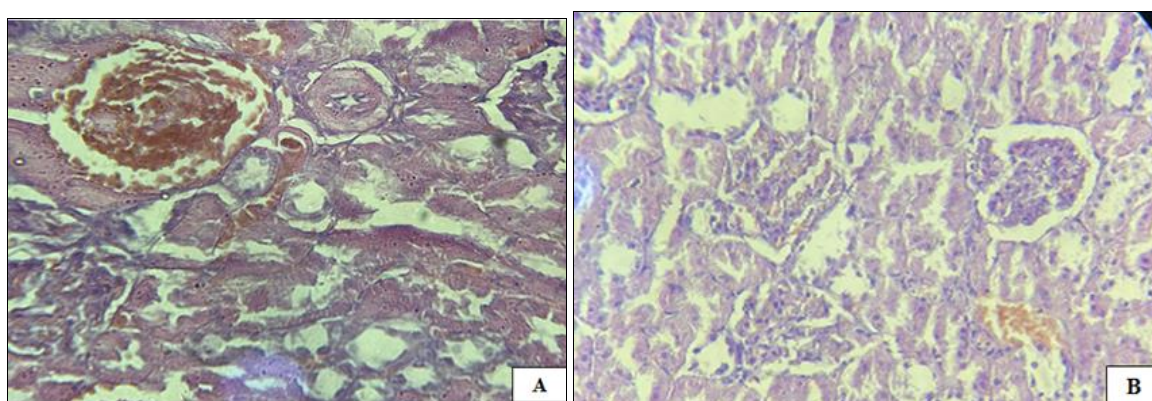


Fig 2: Transvers section of male albino rats Kidney. A: Control, B: Treated group with *L. donovani* for 60 days showing normal appearance of tissue, H&E–stain (X10).

Table 1: Serum total protein levels

Experimental period	Control group (Mean $\pm$ SD, g/dL)	Infected group (Mean $\pm$ SD, g/dL)	Statistical result
30 days	5.50 $\pm$ 0.75	5.58 $\pm$ 0.61	Non-significant, $P \geq 0.05$
60 days	5.043 $\pm$ 1.24	4.603 $\pm$ 0.624	Non-significant, $P \geq 0.05$

Table 2: Serum albumin levels

Experimental period	Control group (Mean $\pm$ SD, g/dL)	Infected group (Mean $\pm$ SD, g/dL)	Statistical result
30 days	3.50 $\pm$ 0.16	3.44 $\pm$ 0.22	Non-significant, $P \geq 0.05$
60 days	2.77 $\pm$ 0.37	2.50 $\pm$ 0.80	Non-significant, $P \geq 0.05$

Note: The thesis reported an overall non-significant albumin comparison ($P = 0.152$).

Table 3: Serum globulin levels

Experimental period	Control group (Mean $\pm$ SD, g/dL)	Infected group (Mean $\pm$ SD, g/dL)	Statistical result
30 days	1.99 $\pm$ 0.697	2.146 $\pm$ 0.815	Non-significant
60 days	2.27 $\pm$ 0.45	1.83 $\pm$ 0.97	Non-significant

Note: The values were taken from the original globulin table. The thesis summary contains an apparent transcription error in which a GPT value was repeated as a globulin value.

Table 4: Summary of kidney histopathological findings

Experimental period	Control group	Infected group
30 days	Normal renal architecture	Focal glomerular atrophy and hyaline casts in renal tubules
60 days	Normal renal architecture	Glomerular congestion and tubular degeneration

Note: The thesis summary described the 60-day kidney as having normal architecture, whereas the detailed figure caption and discussion reported glomerular congestion and tubular degeneration. The detailed result was retained here, but the original slides should be rechecked before journal submission.

Table 5: Relative kidney-weight result

Parameter	30-day comparison	60-day comparison
Relative kidney weight	No significant difference, $P \geq 0.05$	No significant difference, $P \geq 0.05$

Note: The thesis presented organ-weight findings graphically without clearly extractable numerical means.

Interpretive summary: Renal histological alterations were detected despite non-significant changes in total protein, albumin, globulin, and relative kidney weight. These serum proteins are not specific renal-function markers and should not be interpreted as evidence of preserved glomerular filtration.

Discussion

The focal glomerular atrophy and tubular hyaline casts observed at 30 days suggest that the kidney responded to systemic infection even though it is not the primary site of parasite replication. Glomerular atrophy may reflect reduced capillary tuft volume, contraction, ischemic change, or a localized response to inflammatory injury. However, H&E morphology alone cannot differentiate among these possibilities. Hyaline casts may result from concentration and precipitation of filtered proteins or tubular secretory products. Their presence could be associated with transient protein handling disturbances, reduced tubular flow, or epithelial stress, but the study did not include urine testing or special stains to determine cast composition.

At 60 days, glomerular congestion and tubular degeneration may indicate a shift toward microvascular disturbance and sustained epithelial injury. Congestion could be associated with altered renal hemodynamics, endothelial activation, or systemic inflammatory effects. Tubular degeneration may result from impaired perfusion, oxidative stress, inflammatory mediators, or exposure to filtered macromolecules. Similar categories of glomerular and tubular lesions have been described in visceral leishmaniasis and canine leishmaniosis, although species, inoculum, parasite strain, disease severity, and observation interval differ substantially among studies (Rigo *et al.*, 2013; Santos *et al.*, 2013)^[24, 25].

Immune-complex glomerulopathy is one of the most frequently discussed mechanisms of leishmanial renal disease. Persistent antigenemia and polyclonal antibody production can favor circulating immune-complex formation. Deposition within glomeruli may activate complement, recruit inflammatory cells, and alter the filtration barrier. Nevertheless, the current experiment did not use immunofluorescence, electron microscopy, complement staining, or immunoglobulin labeling. Therefore, immune-complex deposition should be regarded as a possible explanation rather than a demonstrated mechanism.

The absence of significant changes in total protein and albumin does not negate renal histological injury. Serum albumin falls only when loss, redistribution, catabolism, or impaired synthesis exceeds compensatory mechanisms. Focal lesions in five animals may be insufficient to produce a detectable group-level biochemical shift. Furthermore, serum protein values are not kidney-specific. Urine protein-to-creatinine ratio, microalbuminuria, serum creatinine, symmetric dimethylarginine, cystatin C, and tubular injury biomarkers would have provided a more direct functional context.

The temporal difference between the 30- and 60-day lesions deserves attention. It should not be interpreted as a simple linear progression because different animals were examined at each time point and no longitudinal biopsy was performed. The 30-day and 60-day groups may represent distinct snapshots influenced by biological variability. Even so, the change from focal atrophy and casts to congestion

and tubular degeneration possibly reflects evolving renal responses during persistent systemic infection.

The lack of kidney enlargement is also unsurprising. Relative organ weight is insensitive to focal microscopic changes. Significant weight alteration generally requires edema, extensive inflammation, cellular proliferation, fibrosis, or parenchymal loss of sufficient magnitude. The present lesions were described microscopically and may not have involved enough tissue to change total organ mass.

From a translational perspective, these findings reinforce the need to consider renal surveillance in visceral leishmaniasis. Human renal involvement ranges from mild urinary abnormalities to acute kidney injury and glomerulonephritis, but direct extrapolation from rats is inappropriate. The experimental dose, intraperitoneal route, and controlled laboratory setting differ from natural sand-fly transmission. Moreover, the rat model may not reproduce the immunological balance of human or canine disease.

Methodologically, the strength of the study lies in its time-matched controls and two observation intervals. Its principal weakness is the descriptive nature of the renal assessment. Without blinded scoring, lesion prevalence, severity, and inter-animal consistency cannot be evaluated. A future kidney-focused protocol should prespecify glomerular, tubular, interstitial, and vascular lesion categories; score multiple fields per animal; and link morphology to functional and immunological markers.

Despite these limitations, the renal observations are not trivial. A structural lesion can have biological significance even when gross organ weight and broad serum proteins are unchanged. The study therefore supports a model in which the kidney is an affected, though not necessarily primary, organ during experimental visceral leishmaniasis. This interpretation is appropriately narrower than a claim of renal failure and better aligned with the available evidence.

The present study also raises a reproducibility question. Histological descriptors such as “focal glomerular atrophy” and “tubular degeneration” may vary among observers unless operational definitions are prespecified. A pathology protocol should define the percentage of shrunken glomeruli, capsular-space changes, epithelial vacuolation, tubular dilation, cast abundance, interstitial inflammation, and vascular congestion. Inter-observer agreement should be tested, particularly when lesion severity is low.

An expanded biochemical panel should include serum creatinine and blood urea nitrogen, but these conventional markers are relatively insensitive to early injury. Cystatin C, urinary NGAL, KIM-1, albumin-to-creatinine ratio, and urine sediment examination could detect subclinical glomerular or tubular dysfunction. Combining these variables with histology would permit a more coherent clinicopathological interpretation.

Future experimental design should distinguish direct parasite localization from secondary immune injury. In situ hybridization, parasite-specific immunohistochemistry, or quantitative PCR could determine whether parasite DNA or organisms are present in kidney tissue. If renal lesions occur without detectable parasite localization, systemic inflammatory or immune-complex mechanisms would become more plausible. If parasites are detected within renal macrophages or endothelial cells, direct local participation could be considered, although the presence of parasite material would still not prove that it caused the lesion.

Renal histopathology in leishmaniasis is especially complex because evidence comes from different host species. Dogs frequently develop chronic immune-complex glomerulopathy during *Leishmania infantum* infection, while human visceral leishmaniasis may produce acute kidney injury through hemodynamic, inflammatory, infectious, or treatment-related pathways. Rodent models vary in susceptibility and immune response. The current rat findings align broadly with renal involvement but should not be forced into a canine or human disease template.

The reported non-significant albumin and total-protein findings should also be interpreted in light of statistical power. With $n = 5$ per group, moderate biological effects may not reach significance, especially when variance is substantial. A non-significant result does not prove equivalence. Formal equivalence testing or confidence intervals would be required to support a conclusion that infected and control groups were meaningfully similar. The present study is therefore best viewed as showing no detected difference rather than demonstrating absence of a biochemical effect.

Another possibility is that systemic dehydration or reduced intake contributed to cast formation and tubular stress. The infected animals experienced significant body-weight reduction, and visceral leishmaniasis can be associated with anorexia and wasting. Reduced fluid intake may concentrate tubular contents, whereas catabolic illness may increase filtered protein fragments. These possibilities are not mutually exclusive with parasite-driven inflammation. Because water consumption, urine volume, and hydration indices were not recorded, the relative contribution of systemic illness cannot be resolved.

Vascular factors also merit consideration. Glomerular congestion at 60 days may reflect increased intraglomerular blood volume or impaired venous outflow, but it can also arise during tissue processing or terminal circulatory changes. Reproducibility across several animals and fields is therefore essential before assigning biological significance. A blinded pathological assessment could classify congestion as absent, mild, moderate, or severe and determine whether it co-localizes with tubular injury. Endothelial markers, fibrin staining, and assessment of peritubular capillaries would clarify whether microvascular injury contributes to the observed pattern.

A further interpretive issue concerns the relationship between glomerular and tubular compartments. The nephron functions as an integrated unit: a change in glomerular permeability can increase the filtered load presented to proximal tubular cells, while tubular epithelial injury can reduce reabsorptive capacity and promote intraluminal cast formation. Thus, glomerular atrophy and hyaline casts at 30 days may represent linked events, but the available data cannot demonstrate that one preceded the other. Segment-specific markers such as megalin, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, and aquaporins would help localize injury and distinguish altered protein handling from nonspecific degeneration.

Finally, publication-quality reporting should follow the ARRIVE 2.0 principles for animal research. This includes specifying allocation methods, housing conditions, humane endpoints, sample-size rationale, blinding, and the number of tissue sections and microscopic fields examined. Such reporting would make the findings easier to reproduce and would strengthen their value for experimental pathology.

Sex is another boundary on interpretation. Only adult males were studied. Hormonal and immunological differences can

influence parasite control, inflammation, vascular responses, and tissue repair. Inclusion of females in future experiments would determine whether the reported organ pattern is sex-dependent. Age and nutritional status should likewise be standardized and reported in detail.

The animal model has practical strengths, including controlled exposure, time-matched controls, and uniform tissue processing. At the same time, natural visceral leishmaniasis is initiated in the skin by metacyclic promastigotes delivered with sand-fly salivary components. Intraperitoneal inoculation bypasses early dermal and vector-associated events and may alter dissemination kinetics. Therefore, the model is useful for studying systemic organ responses but should not be presented as an exact reproduction of natural infection.

Interpretation should also account for the exploratory nature of the experiment. The study was not originally powered around a single-organ primary endpoint, and multiple physiological and histological outcomes were examined. Consequently, the findings are most appropriately treated as hypothesis-generating. Replication in an independently powered experiment is needed before estimating the magnitude or frequency of the reported lesions. Publication should include raw individual-animal values, a clear statement of randomization and blinding procedures, and transparent reporting of any excluded specimens.

Conclusion

Experimental *Leishmania donovani* infection produced distinct renal histological abnormalities in male albino rats. Focal glomerular atrophy and tubular hyaline casts were observed at 30 days, whereas glomerular congestion and tubular degeneration were observed at 60 days. These lesions occurred without significant changes in relative kidney weight or the measured serum protein variables. The findings support the presence of renal tissue involvement but do not establish renal failure, immune-complex deposition, direct parasitism of renal cells, or a definitive causal pathway.

Recommendations

Measure serum creatinine, urea, electrolytes, cystatin C, and urinary protein indices.

Use blinded semiquantitative histopathological scoring and morphometry.

Apply PAS, Masson trichrome, immunofluorescence for immunoglobulins/complement, and parasite immunohistochemistry or PCR.

Increase sample size and include additional time points.

Assess oxidative stress, renal cytokines, and endothelial markers.

Limitations of the Study

The study included only five animals per group, limiting statistical power and the ability to characterize biological variability. Histology was primarily descriptive and was not supported by lesion scoring, morphometry, immunohistochemistry, electron microscopy, or quantitative parasite detection. Kidney-specific functional markers and urinalysis were not performed. The use of intraperitoneal inoculation does not reproduce natural vector transmission. Finally, the study used separate cohorts at 30 and 60 days, so temporal progression was inferred from group comparisons rather than followed longitudinally in the same animals.

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