Research Article

Immunological and Histopathological Evaluation of Selenium Nanoparticles in Rabbits Infected with *Eimeria stiedae* (Hepatic Coccidiosis)

Tuqa Najim Al-Waely*[1], Haider Mohammed Ali Al-Rubaie[1] and Laith Ahmed Yaaqoob[2]

[1]Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; [2]Department of Biotechnology, College of Science, University of Baghdad, Iraq.

Abstract | Hepatic coccidiosis caused by *Eimeria stiedae* leads to hepatic dysfunction and severe biliary hyperplasia in rabbits, and its treatment remains challenging due to drug resistance and concerns about chemical residues. Vaccination with sonicated antigens provides only partial protection, while selenium nanoparticles (Se-NPs), known for their antioxidant and immunomodulatory properties, may enhance antigen efficacy. This study aimed to evaluate whether green-synthesized Se-NPs, prepared from Na_2SeO_4 and an aqueous extract of clove (*Syzygium aromaticum*), can improve immune responses and mitigate hepatic histopathological damage following experimental infection in local rabbits. Characterization of Se-NPs revealed a mean particle size of 65.96 nm and a stable negative surface charge with a zeta potential of −28.40 mV. Immunological analysis showed that the Ag + Se-NPs group exhibited the highest IgG concentration (17.42 ± 1.07 µg/mL), followed by the positive control group (13.73 ± 0.87 µg/mL). Furthermore, adenosine deaminase (ADA) activity was significantly modulated in the Ag + Se-NPs group, decreasing to 761.14 ± 25.64 pg/mL approaching levels observed in the negative control compared with peak activity in the positive control group (1552.01 ± 71.92 pg/mL). Histopathological examination demonstrated that the Ag- and Se-NPs-treated groups exhibited minimal bile duct hyperplasia, reduced epithelial desquamation, and markedly fewer parasitic developmental stages. In contrast, the positive control group showed severe ductal proliferation and granulomatous inflammation. In conclusion, Se-NPs act as an effective carrier for sonicated *E. stiedae* antigen, enhancing immune protection and reducing hepatic lesions. To the best of our knowledge, this is the first study to investigate the combined effects of Se-NPs and sonicated antigens on hepatic coccidiosis in local rabbit breeds, particularly with respect to ADA enzyme activity.

Keywords | *Eimeria stiedae*, Hepatic coccidiosis, Selenium nanoparticles, Sonicated antigen, Rabbits, Adenosine deaminase (ADA)

Received | March 03, 2026; **Accepted** | August 04, 2026; **Published** | August 31, 2026
***Correspondence** | Tuqa Najim Al-Waely, Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Iraq; **Email:** tuqa.najm2205p@covm.uobaghdad.edu.iq
Citation | Al-Waely TN, Al-Rubaie HMA, Yaaqoob LA (2026). Immunological and histopathological evaluation of selenium nanoparticles in rabbits infected with *Eimeria stiedae* (Hepatic Coccidiosis). J. Anim. Health Prod. 14(3): 1217-1230.
DOI | https://dx.doi.org/10.17582/journal.jahp/2026/14.3.1217.1230
ISSN (Online) | 2308-2801

INTRODUCTION

Eimeria stiedae-induced hepatic coccidiosis is a serious parasitic infection of the rabbit liver, which chiefly originates upon the bile-duct epithelium (Abd El-Ghany, 2020). It is associated with cholangitis, bile duct hyperplasia, hepatomegaly, and impaired growth, and is often fatal, posing a significant threat to rabbits' health and productivity (Steffen *et al.*, 2025). Typical gross lesions include a large, pale liver with nodules and a cream-white colour, and distended gallbladders; however, histopathological observations reveal distinctive bile-duct

ectasia and proliferation of epithelial cells with numerous parasitic stages (Al-Naimi *et al*., 2012; Hegazi *et al*., 2023).

The growing resistance to traditional anticoccidials, combined with concerns about chemical residues in meat, has led to efforts to develop alternative treatment approaches (Kadhim *et al*., 2018; Bharti *et al*., 2025).

There is a case of promising immunotherapeutic approaches that use nanoparticles to deliver antigens as antigen-delivery system immunotherapies, which have demonstrated elevated immunogenicity and reduced toxicity (Liu *et al*., 2019). Selenium nanoparticles (Se-NPs) have strong antioxidant, immunomodulatory, and hepatoprotective effects (Ansari *et al*., 2024). Se-NPs can enhance antigen recognition, enhance immune activation, and reduce tissue destruction when used with specific antigens (Chen *et al*., 2022). They have been of growing interest due to their bioactivity and safety profile in the treatment of parasitic diseases (Sun *et al*., 2019; Metwally *et al*., 2022).

Although the potential of individual constituents has been demonstrated, little research has examined the synergistic application of selenium nanoparticles (Se-NPs) and sonicated antigens for hepatic coccidiosis in local rabbit breeds, specifically regarding the activity of adenosine deaminase (ADA). In turn, the current study aimed to assess the synergistic in vivo biological activity of green-synthesized selenium nanoparticles in combination with sonicated antigens, specifically by associating particular immune responses with histopathological modifications, providing a solid basis for further clinical trials.

MATERIALS AND METHODS

Sample collection

Twenty-eight rabbits were gathered from farms in Baghdad and local markets. *E. stiedae* oocysts were isolated and identified in the Parasitology Laboratory, University of Baghdad, College of Veterinary Medicine.

Synthesis of selenium nanoparticles

For the synthesis of selenium (Se) nanoparticles the reduction and stabilization agent was selected as clove (*Syzygium aromaticum*) extract due to the presence of a large amount of phenolic compounds and the proven antioxidant properties in accordance with the ability of a green synthesis of behaviour resembling the biological effect of selenium nanoparticles., 10g of Na_2SeO_4 powder was added to a flask containing 100 mL of an aqueous clove extract. The mixture was shaken in a digital orbital shaker (WiseShake® SHO-2D) in the darkroom overnight. The mixture was centrifuged at 5000 rpm for 10 minutes. The precipitate from a solution containing all the selenium nanoparticles was washed with ethanol. The sample was centrifuged at 5000 rpm, and 5 mL of deionized distilled water (DDW) was added. The solution was transferred to a glass Petri dish and incubated at 40 °C for 72 hours (Yaaqoob *et al*., 2022). The dark brown Se-NPs powder was stored in a dark container for further characterization and use.

Antigen quantification and immunization

The total protein concentration of the sonicated *E. stiedae* antigen was determined to be 20 mg/mL using an automated analyzer (Cobas e 411, Roche Diagnostics). For immunization, the antigen was diluted to a final working concentration of 2 mg/mL. Each rabbit received a subcutaneous injection of 1 mL of this preparation, delivering a total standardized protein dose of 2 mg per animal.

Experimental animals

Sixty local-breed male rabbits (16–20 weeks old; 1000-1500 g) were sourced from the Baghdad markets and housed in the Animal House, College of Veterinary Medicine, University of Baghdad. Animals were fed fresh vegetables and had free access to water. The 60 rabbits were randomly allocated into six equal groups (n=10 per group). All rabbits were screened by fecal examination to confirm the absence of coccidian oocysts. Rabbits were acclimated for 14 days under controlled conditions. Animals were divided into six groups: G1 received subcutaneous sonicated antigen (Ag) at 1mg/ml combined with 0.1 ml/kg intraperitoneal (IP) selenium nanoparticles (Se-NPs); G2 received subcutaneous Ag. After 14 days, both groups received booster doses according to the same immunization schedule, with G1 also receiving an additional IP Se-NP booster dose. Groups (G3–G5) received 1ml PBS injections, while G6 served as the negative control. On day 22, all groups except G6 were challenged orally with 10^3 sporulated *E. stiedae* oocysts. Seven days post-infection, G3 was treated with 0.1 ml/kg Se-NPs IP, and the treatment was repeated after 10 days, whereas G4 received diclazuril orally at 2 mg/kg B.W, which was repeated after 10 days (Allam *et al*., 2020). On day 38, oocyst shedding was monitored in G1–G5 at 3-day intervals for 16 days. There was adequate statistical power, as the sample size (n=10 per group) was determined according to the laid-down procedures for immunological studies in rabbits. The doses of sonicated antigen (1 mg/ml) administered were based on prior dose-response experiments (Al-Taee and Al-Zubaidi, 2017) to achieve optimal immunogenicity without causing systemic toxicity.

Atomic force microscopy (AFM)

The morphological and surface topographical characteristics of the synthesized Se-NPs were evaluated using an Atomic Force Microscopy (AFM) system (FlexAFM, model

C-3000, Nanosurf AG, Switzerland). This technique was employed to examine critical structural parameters, including particle size distribution, surface roughness, granularity index, and three-dimensional (3D) topography, providing a high-resolution map of the nanoparticle surfaces (Abdul-Hamead, 2020).

Energy dispersive X-ray (EDX) analysis

Elemental composition was determined using Energy Dispersive X-ray (EDX) analysis integrated with the SEM system. The EDX spectrum was recorded by focusing the electron beam on specific regions of interest within the nanomaterial, enabling selective analysis of particular structures or features by comparing distinctive peaks with reference patterns (Ashrafi-Saiedlou *et al*., 2025).

Zeta potential

The electrokinetic potential, commonly known as the zeta potential, was measured to quantify the electrical charge of the colloidal dispersions. Measurements were conducted using a Zeta Potential Analyzer (Zeta Plus, Brookhaven, USA) at the Ministry of Higher Education and Scientific Research. The analysis focused on determining the potential difference between the dispersion medium and the stationary fluid layer attached to the dispersed particles, specifically within the region bounded by the slipping plane.

Infection of animals

Sporulated (10^{3}) *E. stiedae* oocysts were administered orally to induce hepatic coccidiosis. Rabbits were monitored daily for clinical signs and general condition.

Treatment of animals

Treatments included sonicated antigen, selenium nanoparticles, the Se-NP–antigen, and the standard anticoccidial drug (diclazuril). Doses were administered according to the designed experimental schedule.

Immunological parameters

Serum IgG levels were quantified, and adenosine deaminase (ADA) activity was measured using appropriate ELISA kits (Sunlong Biotech Co., Ltd., Hangzhou, China; Cat. No: SL009Rb and SL0345Rb, respectively).

Histopathological examination

At day 55 post-challenge, rabbits were euthanised gently and sacrificed. Livers and gallbladders were examined for lesion scoring (hepatomegaly, nodules, congestion, bile duct thickening). Samples were fixed in 10% buffered formalin, processed, embedded in paraffin, sectioned (4–5 µm), and stained with Hand E. Lesion grading was performed according to established descriptions of *E. stiedae* pathology and subsequently analysed by light microscopy (Talab and Falih, 2026).

Statistical analysis

Analysis of data via Two-Way Analysis of Variance (ANOVA) in SPSS (version 26, 2019) was carried out. The model accounted for the effects of different treatment groups and time points (repeated measures) on the study parameters. The means were compared for significant differences using the Least Significant Difference (LSD) post hoc test. P-values < 0.05 were considered significant.

RESULTS

Characterization of nanoparticles

Atomic force microscope (AFM): The AFM analysis confirmed that most particles were nanoscale (<100 nm), with a calculated mean particle size of 65.96 nm, as detailed in Table 1. Furthermore, the 3D topography (Figure 1) revealed a notably rough surface profile, which is characteristic of the synthesized Se-NPs.

Table 1: EDX spectrum of Selenium nanoparticles.

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
O	69.7	2.2	30.8	1.0
Se	30.3	1.3	69.2	3.0

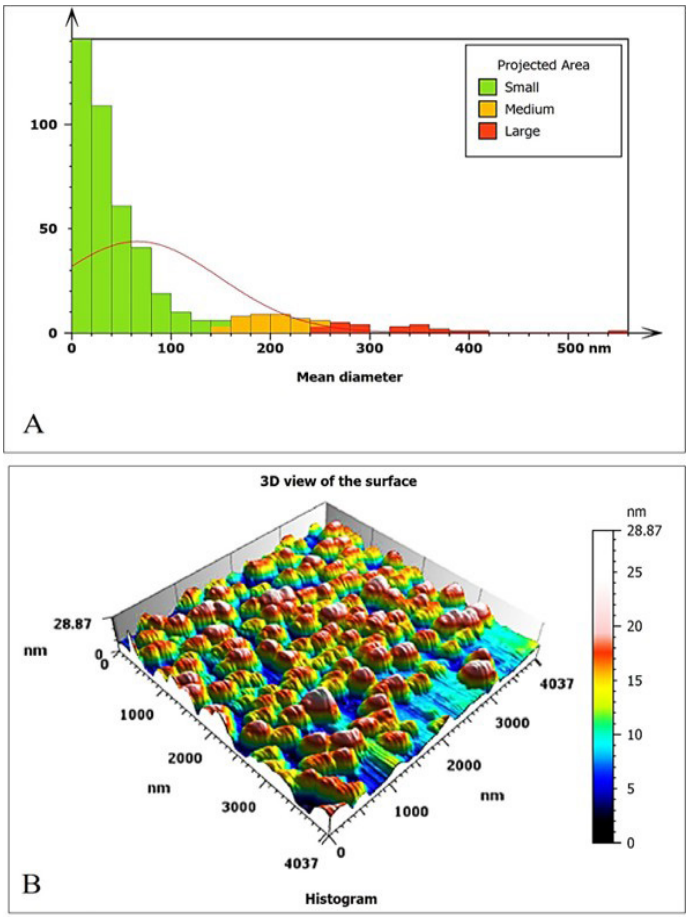

Figure 1: A: Selenium granularity cumulation distribution chart (Histogram); B: AFM 3D image of Se-NPs nanoparticles.

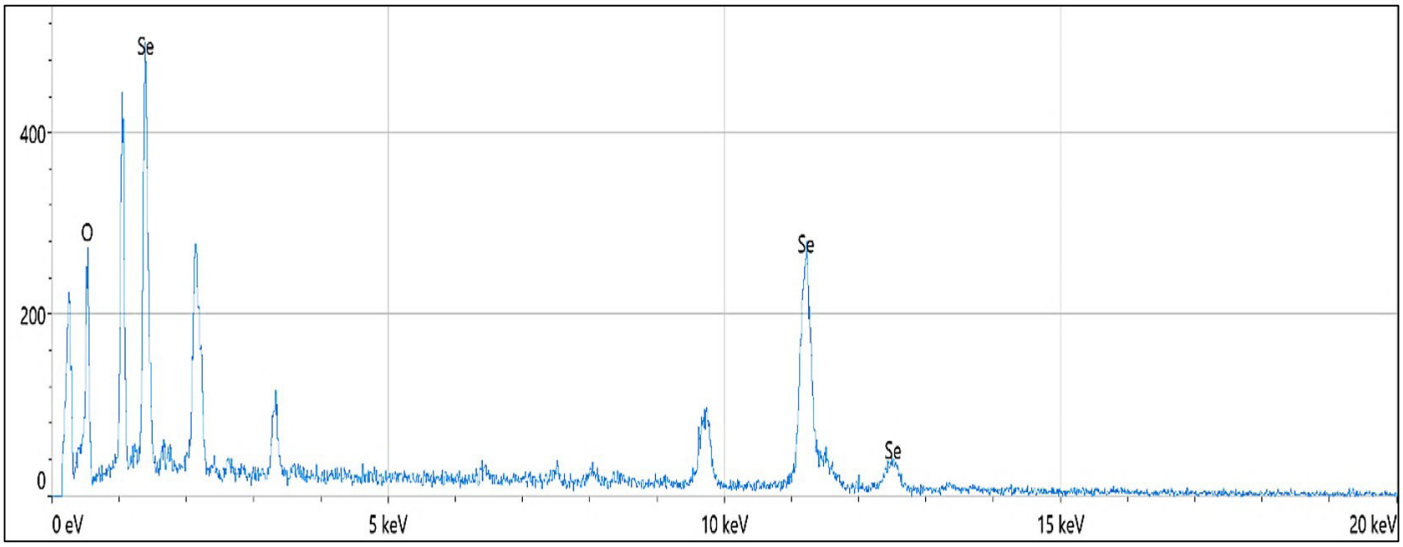

Figure 2: EDX spectrum of Se nanoparticles.

Energy-dispersive X-ray spectroscopy (EDX) analysis

The characteristic absorption peak of selenium is observed at 30 kV, and selenium accounts for the highest percentage of total elements, at a ratio of 69.2% (Table 2 and Figure 2).

Table 2: Dimensions of prepared Selenium nanoparticles.

Nanoparticle	Average value (nm)	Minimum (nm)	Maximum (nm)
Se-NPs	65.96	5.86	306

Zeta potential

Se-NPs green synthesized in the present study were confirmed to have a negative surface charge with a zeta potential value of -28.40 mV (Figure 3).

Immunoglobulin G (IgG)

IgG levels varied significantly among the groups ($P < 0.05$), with a least significant difference (LSD) value of 3.893. The Ag and Se-NPs group recorded the highest IgG concentration (17.42 ± 1.07 µg/mL), exceeding those of most other groups, indicating a substantial enhancement of antigen-specific humoral immunity when selenium nanoparticles were combined with the *E. stiedae* antigen. The Ag group also shows elevated IgG (15.65 ± 0.62 µg/mL), but to a lesser extent than the IgG in the IgG group. Se-NPs (12.37 ± 0.57 µg/mL) and diclazuril (11.01 ± 0.41 µg/mL) produced significantly lower IgG responses, consistent with weaker immunostimulatory effects in the absence of antigenic priming. The positive control displayed a moderate IgG rise (13.73 ± 0.87 µg/mL), whereas the negative control had the lowest baseline IgG (9.25 ± 0.62 µg/mL) (Figure 4A).

Adenosine deaminase (ADA)

ADA activity showed a distinct pattern across groups ($P < 0.05$), with an LSD value of 208.62. The positive control exhibited the highest ADA value (1552.01 ± 71.92 pg/mL), indicating intense cellular immune activation and inflammatory response to ongoing infection. Elevate ADA is also observed in Se-NPs (1368.36 ± 75.94 pg/mL) and Ag groups (1272.67 ± 81.22 pg/mL), followed by diclazuril (1187.19 ± 60.43 pg/mL). In contrast, the Ag + Se-NPs group shows markedly reduced ADA activity (761.14 ± 25.64 pg/mL), which was close to that of the negative control (674.35 ± 34.12 pg/mL) (Figure 4B).

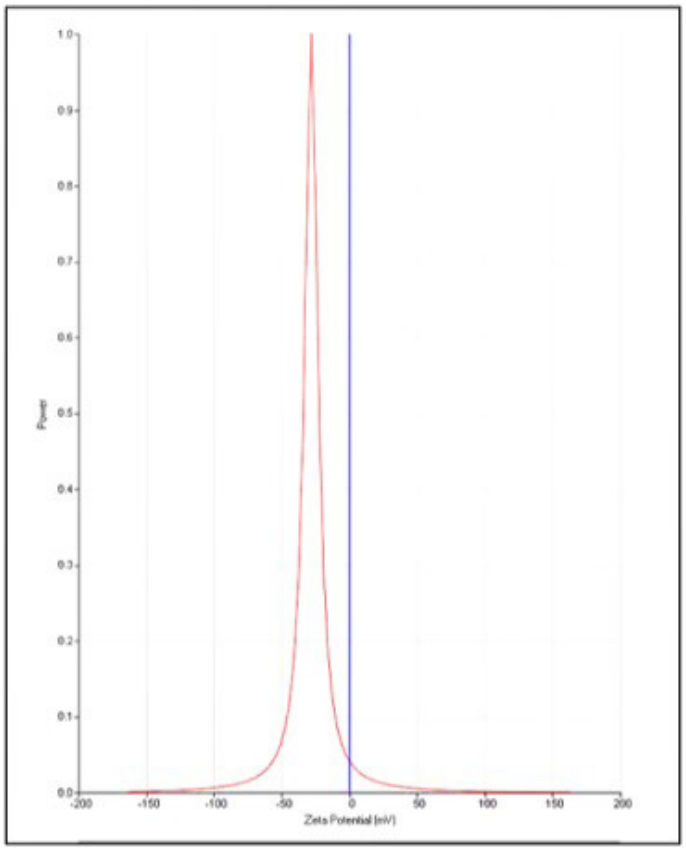

Figure 3: Zeta potential of Se nanoparticles.

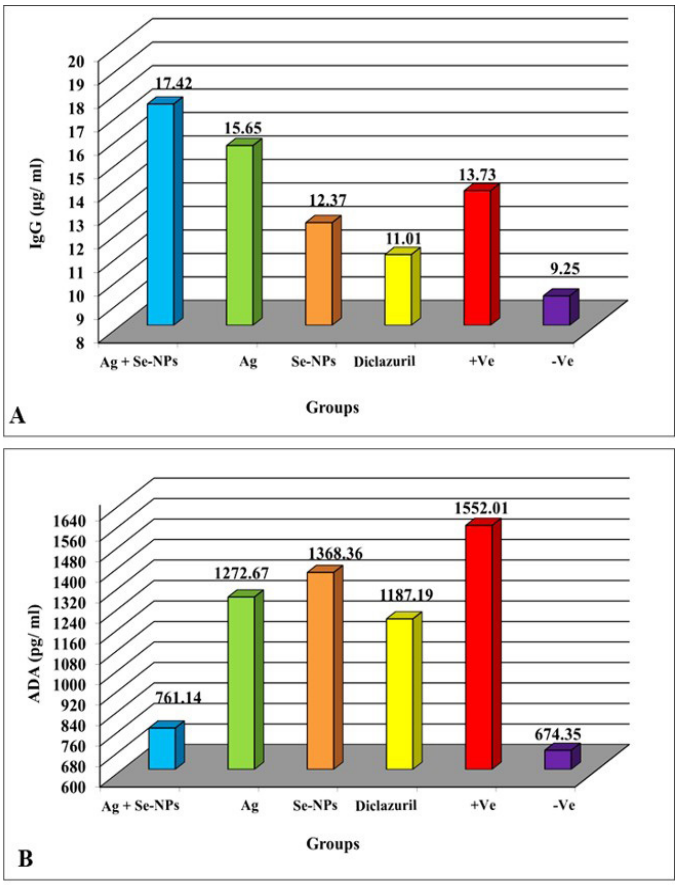

Figure 4: Comparison between different groups of E. stiedae infection in: (A) Immunoglobulin G (IgG), (B) Adenosine Deaminase (ADA). Bars represent the mean ± SE. Different superscript letters (a, b, c) indicate statistically significant differences between groups at $P < 0.05$.

Gross lesions

Grossly, *E. stiedae* infection in the positive control group caused hepatomegaly, discolouration, and nodular lesions filled with caseous material, as well as distended, bile-filled gallbladders. In contrast, the Ag + Se-NPs group showed only mild congestion and no typical hepatic coccidial lesions, and the Ag and diclazuril groups showed partial protection. In contrast, Se-NPs alone conferred the least improvement, yet it was still evident relative to untreated infected rabbits (Figure 5).

Histopathological lesions

Histopathological evaluation clearly demonstrated that *E. stiedae* infection induced severe hepatic and gallbladder lesions, characterized by marked bile duct hyperplasia, periductal fibrosis, and dense inflammatory cell infiltration (Figures 6, 7). The combined administration of antigen and Se-NPs yielded the most pronounced protective effect, with the liver and gallbladder tissues appearing nearly normal (Figure 8). Immunization with the *E. stiedae* antigen resulted in partial protection, with reduced hepatobiliary changes and parasitic burden (Figure 9). Treatment with diclazuril also alleviated considerable pathology (Figure 10). In contrast, treatment with selenium nanoparticles (Se-NPs) alone resulted in only a mild improvement, with a partial reduction in bile duct proliferation (Figure 11).

Figure 5: Gross morphological examination of liver and gallbladder across experimental groups: (A) Negative control showing normal reddish-brown color and sharp margins. (B) Positive control exhibiting severe hepatomegaly and disseminated "milk-spot" nodules. (C) The Ag and Se-NP-treated group showed congested parenchyma with a smooth capsule and no nodules. (D) Sonicated antigen group showing mild enlargement and scattered small nodules (green arrow). (E) The diclazuril-treated group presented mild hepatomegaly and sparse white nodules (green arrow). (F) The Se-NPs group showed moderate hepatomegaly and discrete nodules (green arrow) with mild icterus.

DISCUSSION

The Se-NPs synthesis using sodium selenate (Na_2SeO_4) and clove (*Syzygium aromaticum*) extract exhibited a mean AFM size of ~66 nm. This result is consistent with that of Li *et al.* (2008), who reported Se nanoplatelets with an average diameter of approximately 60 nm. EDX is used to confirm that the reduction product is a nano selenium particle. Furthermore, analysis indicates that the main component of the reduction product is selenium, consistent with the findings of Saravanan *et al.* (2025). Zeta Potential results are consistent with those reported by Puri and Patil (2022), who found a value of -28.1 mV. Moreover, Vekariya *et al.* (2012) reported a zeta potential of -28.8 mV. Nevertheless, Sentkowska *et al.* (2025) demonstrated that the Se-NPs exhibit high stability, as confirmed by their negative zeta potential value of -29.4 mV.

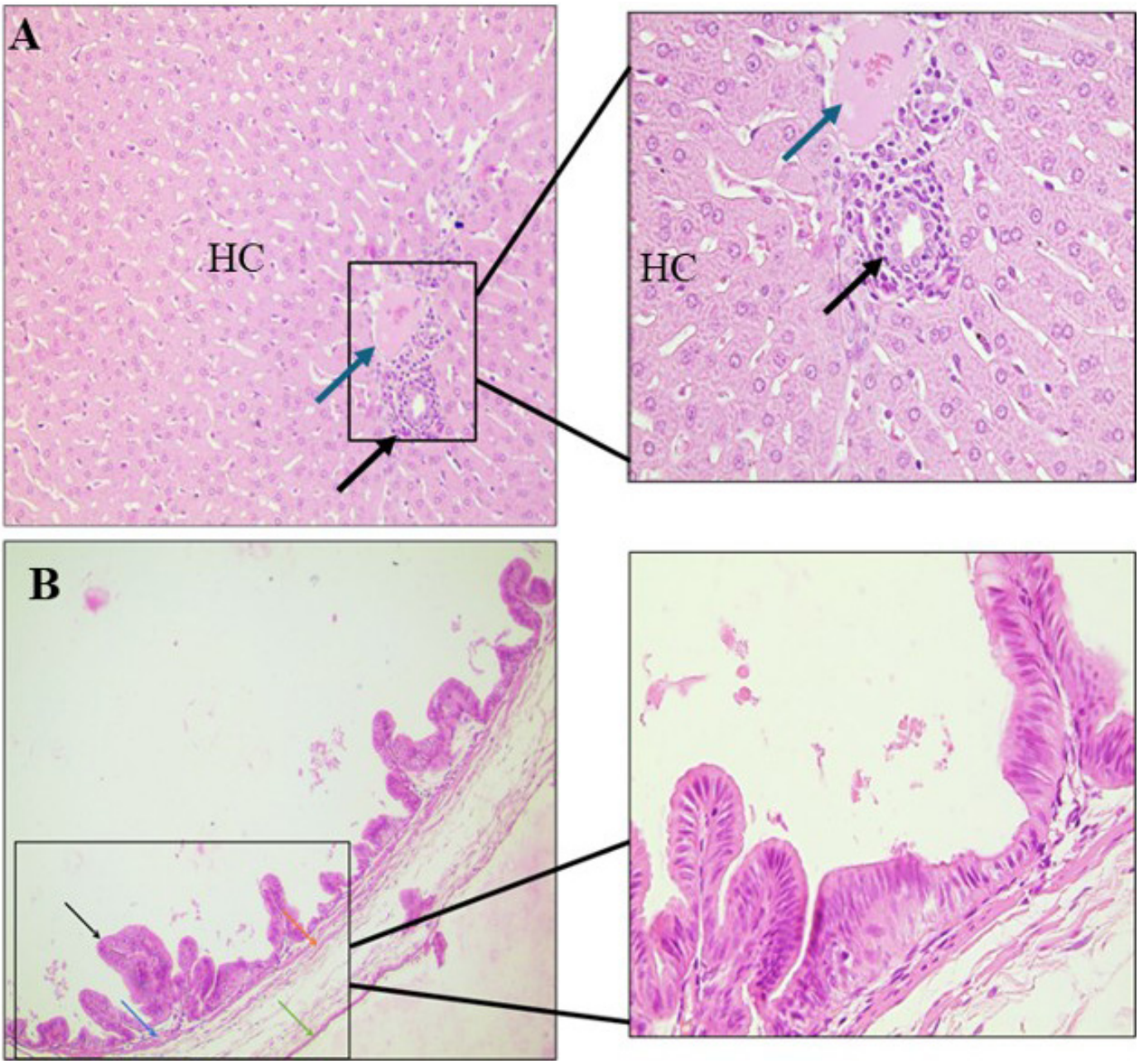

Figure 6: Histological section of the liver and gallbladder of rabbits of the negative control group: (A) Liver section shows normal parenchyma with a typical central vein (CV), hepatic cord (HC), bile duct (black arrow), and portal vein (blue arrow). (B) The gallbladder section shows an empty lumen (L) with normal wavy mucosal folds lined by simple columnar epithelium (black arrow). The wall architecture consists of normal submucosa (blue arrow), muscular layer (orange arrow), and serosa (green arrow). (H and E. 10,40X).

In the present study, co-administration of *E. stiedae* antigen with selenium nanoparticles (Ag + Se-NPs) was associated with a marked increase in serum IgG, alongside a reduction in ADA activity compared with infected positive controls. This pattern suggests that Se-NPs act as adjuvants that amplify antigen-specific humoral immunity while tempering excessive cell-mediated inflammatory responses, indicating a dual immunomodulatory and protective role.

Eimeria stiedae experimental infections have demonstrated that parasite-specific IgG is already detected 4 days after infection and reaches high levels over the course of the following weeks, with much higher levels being observed between 2 and 4 weeks of exposure (Cam *et al*., 2008; Al-Taee and Al-Zubaidi, 2017; Hegazi *et al*., 2023). Other *Eimeria* vaccines have reported similar IgG kinetics in rabbits, in which immunisation results in both persistent increases in specific IgG, which correlate with a reduction in oocyst output and an amelioration of liver lesions (Awade *et al*., 2019). Thus, the enhanced IgG response in the Ag + Se-NP group is consistent with effective priming of the adaptive immune response. It likely contributes to improving clinical and parasitological outcomes observed in our experiment.

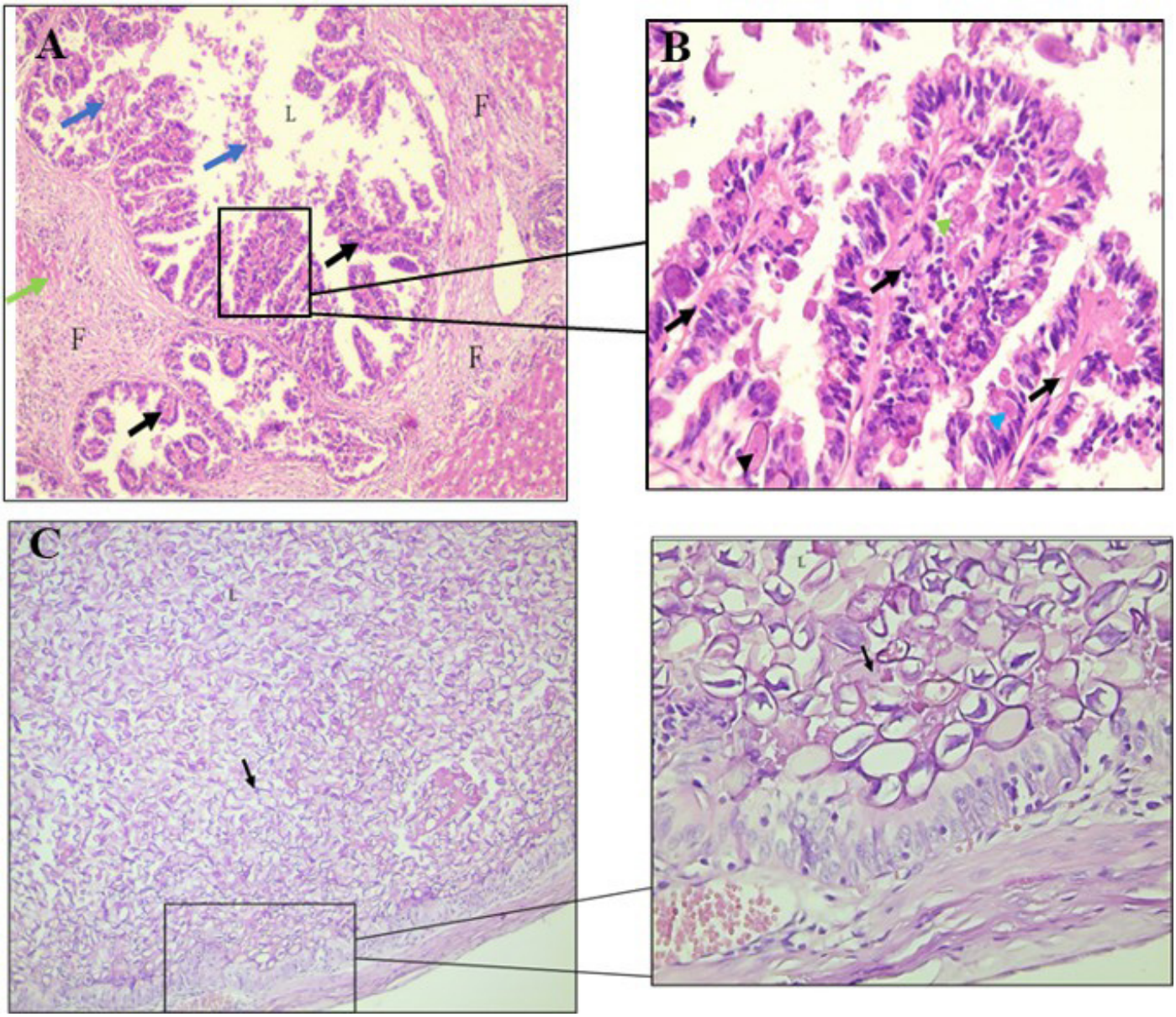

Figure 7: Histopathological section of the liver and gallbladder of the positive control group: (A) Liver section shows dilated bile ducts and epithelial hyperplasia with papillary projections (black arrows) toward the lumen (L), characterized by necrotic and desquamative lining with developmental stages of E. stiedae (E). Extensive periductal fibrosis with mononuclear cell infiltration (F) and severe hepatocyte necrosis (green arrow) are evident. (B) High magnification reveals numerous stages embedded in the epithelium: macrogametes (green arrowhead), microgametes (blue arrowhead), and oocysts (black arrowhead). (C) The gallbladder section shows a highly dilated lumen (L) with flattened epithelium, containing a large number of oocysts (black arrow). (H and E, 10X, 40X).

Adenosine deaminase levels increased significantly above baseline post-infection. A report in goats infected with *Eimeria arloingi* noted that ADA activity increased from near-normal baseline levels on day 0 to peak levels by day 7–14 post-infection, and it remained low in uninfected controls (Tadayon *et al.*, 2016). By analogy, rabbits with hepatic coccidiosis are likely to show elevated serum ADA levels as the infection progresses, and this rise in ADA correlates with the onset of robust cell-mediated immunity to *Eimeria* (Asherson and Rose, 1963). Elevated ADA levels have also been reported in experimental toxoplasmosis models, in which *T. gondii* infection significantly upregulates its activity in T lymphocytes and brain tissues, reflecting an intense Th1-type cellular response (Tonin *et al.*, 2013). Conversely, some protozoal infections, such as bovine cryptosporidiosis, have been associated with decreased serum ADA, underscoring that a modulation is pathogen- and host-dependent but generally mirrors the intensity and quality of the cellular immune response (Yarim *et al.*, 2016). Beyond protozoa, it is increasingly used as a nonspecific inflammatory and immunological biomarker across domestic species, with well-characterized reference values in cattle, small ruminants, dogs and horses (Contreras-Aguilar *et al.*, 2020).

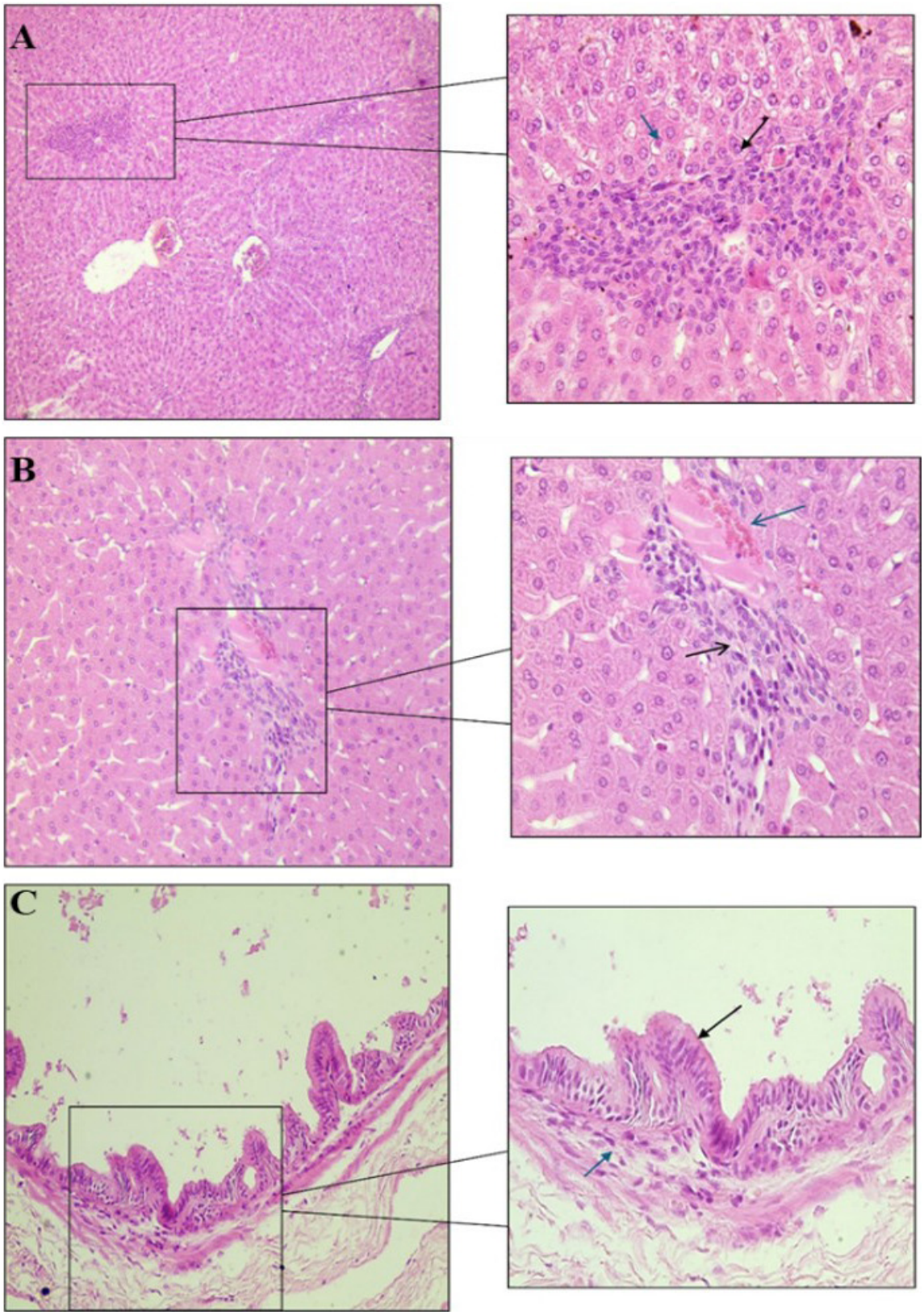

Figure 8: Histopathological section of the liver and gallbladder of the Se-NPs and antigen group: (A) Liver section showing a localized, dense lymphocytic aggregate between hepatic plates (black arrow) associated with mild hepatocellular degeneration. Most hepatocytes remain intact, while adjacent cells exhibit cytoplasmic granularity (blue arrow) and mild sinusoidal dilatation. (B) Liver section showing preserved lobular architecture with well-defined foci of lymphocytic inflammation and mild hepatocellular swelling (black arrow). Sinusoids are mildly congested (blue arrow), consistent with mild focal hepatitis and early immune activation. (C) Gallbladder section showing mild villous oedema and elongation of mucosal folds (black arrow) with mild epithelial hyperplasia. The lamina propria is expanded with lymphocytic infiltration (blue arrow), alongside focal irregularity of the apical border. (H and E, 10X, 40X).

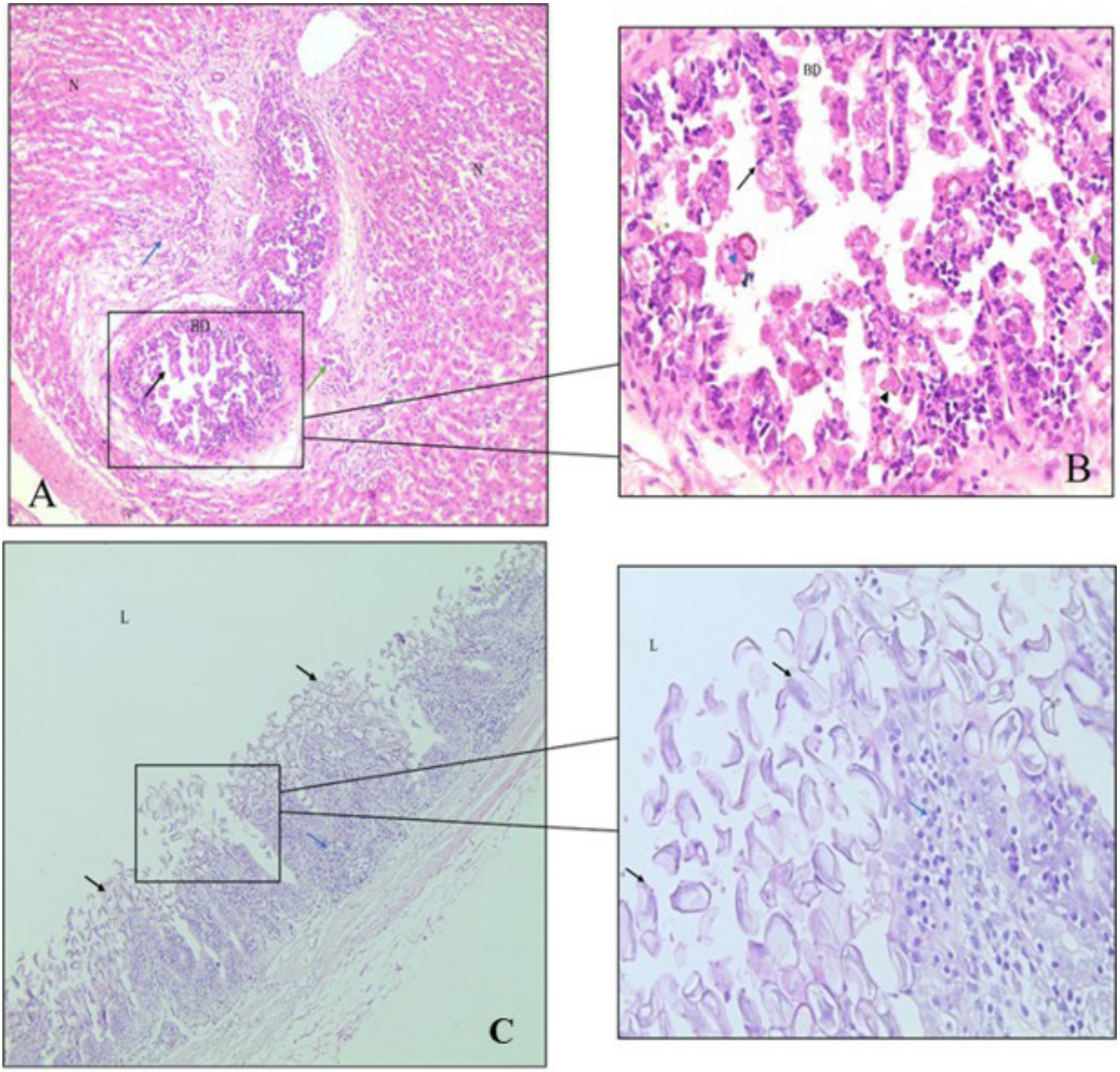

Figure 9: Histopathological section of the liver and gallbladder of the antigen group: (A) Liver section shows mild enlargement of the bile duct (BD) and mild epithelial hyperplasia with papillary projections (black arrow) containing few developmental stages of E. stiedae. Note the multifocal periductal fibrosis with mononuclear cell infiltration (blue arrow), bile duct hyperplasia (green arrow), and mild to moderate hepatocellular necrosis (N). (B) High magnification reveals a few developmental stages: macrogametes (green arrowhead), microgametes (blue arrowhead), and oocysts (black arrowhead) associated with mononuclear infiltration. (C) The gallbladder section shows a mildly dilated lumen (L) containing a few oocysts (black arrows) with mononuclear cell infiltration (blue arrow). (H and E, 10X, 40X).

To date, no prior reports specifically quantifying ADA activity in rabbits with hepatic coccidiosis, suggesting that the present work provides the first baseline description of ADA dynamics in this host–parasite system.

The gross findings are pathognomonic of hepatic coccidiosis and align with previous literature, which consistently associates the infection with biliary hyperplasia and nodular hepatic lesions (Sivajothi *et al*., 2016; Hegazi *et al*., 2023).

In this study, liver and gallbladder histopathological sections from negative control rabbits show normal findings, with hepatocytes arranged in regular cords around a central vein, intact sinusoids, and unaltered bile ducts and gallbladder mucosa. These findings are consistent with

previous descriptions of normal rabbit liver (Nadia *et al*., 2012; Rafid *et al*., 2015; Sorour *et al*., 2018). In contrast, untreated infected rabbits exhibited hepatic coccidial lesions (marked bile duct hyperplasia with papilliform epithelial projections containing multiple schizonts, gamonts, and oocysts, accompanied by dense periportal mononuclear infiltrates, hepatocellular degeneration/ necrosis, and inflammatory thickening of the gallbladder mucosa). These alterations are consistent with lesions in naturally and experimentally infected rabbits, in which bile duct epithelial hyperplasia, portal inflammation, and hepatocellular necrosis are the typical features of *E. stiedae* infection. This aligns with the findings of Athanasiou *et al*. (2023) and Steffen *et al*. (2025).

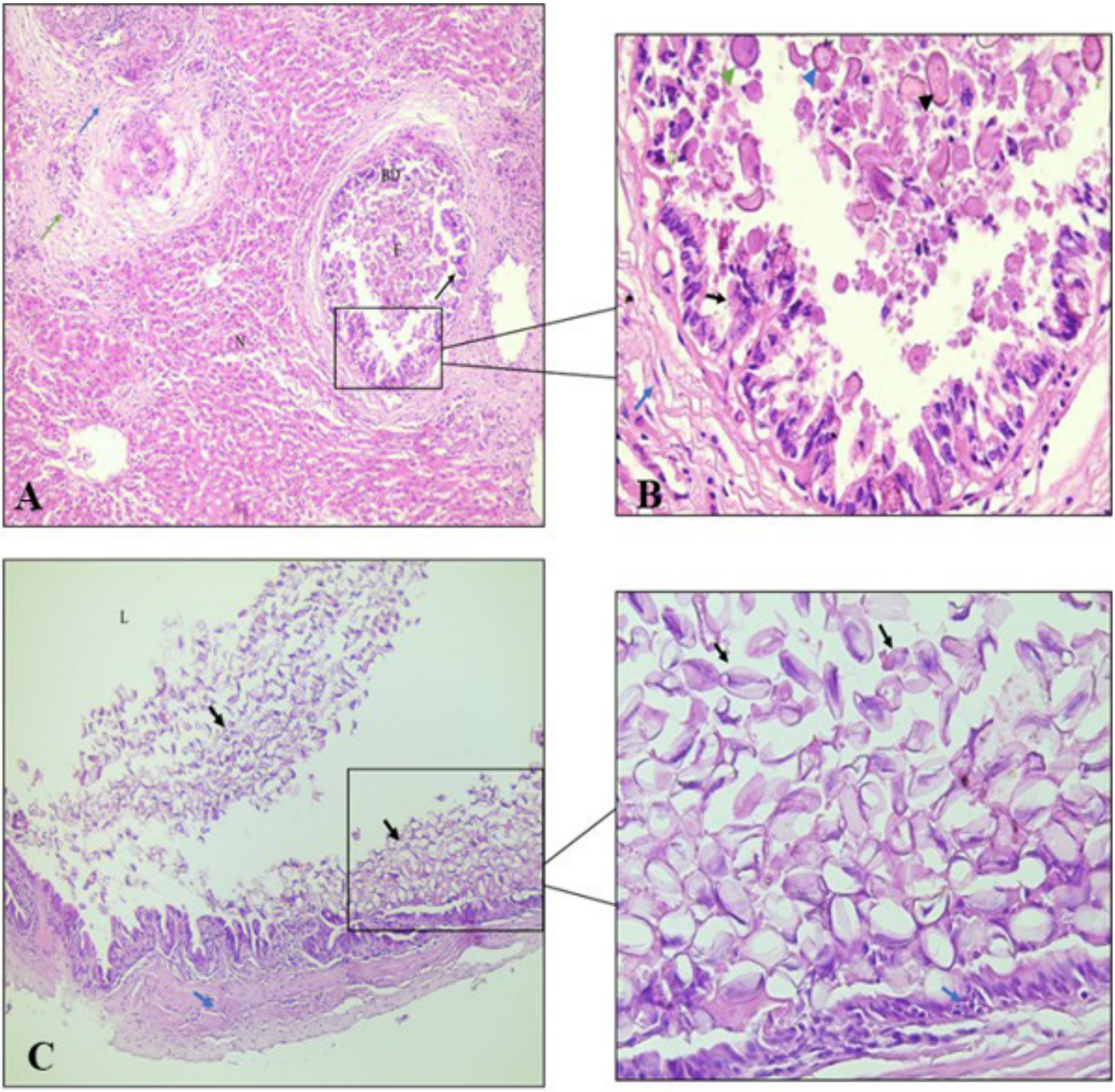

Figure 10: Histopathological section of the liver and gallbladder of the diclazuril-treated group: (A) Liver section shows mild bile duct enlargement with mild-to-moderate epithelial hyperplasia and papillary projections (black arrow) containing moderate developmental stages of E. stiedae (E). Note the multifocal periductal fibrosis with mononuclear cell infiltration (blue arrows), newly formed bile ducts (green arrow), and mild-to-moderate hepatocellular necrosis. (B) High magnification reveals moderate numbers of macrogametes (green arrowhead), microgametes (blue arrowhead), and oocysts (black arrowhead) associated with mild periductal fibrosis (blue arrow). (C) Gallbladder section shows a mildly dilated lumen (L) with flattened epithelium, containing a moderate number of oocysts (black arrow) and mononuclear cell infiltration (blue arrow). (H and E, 10X, 40X).

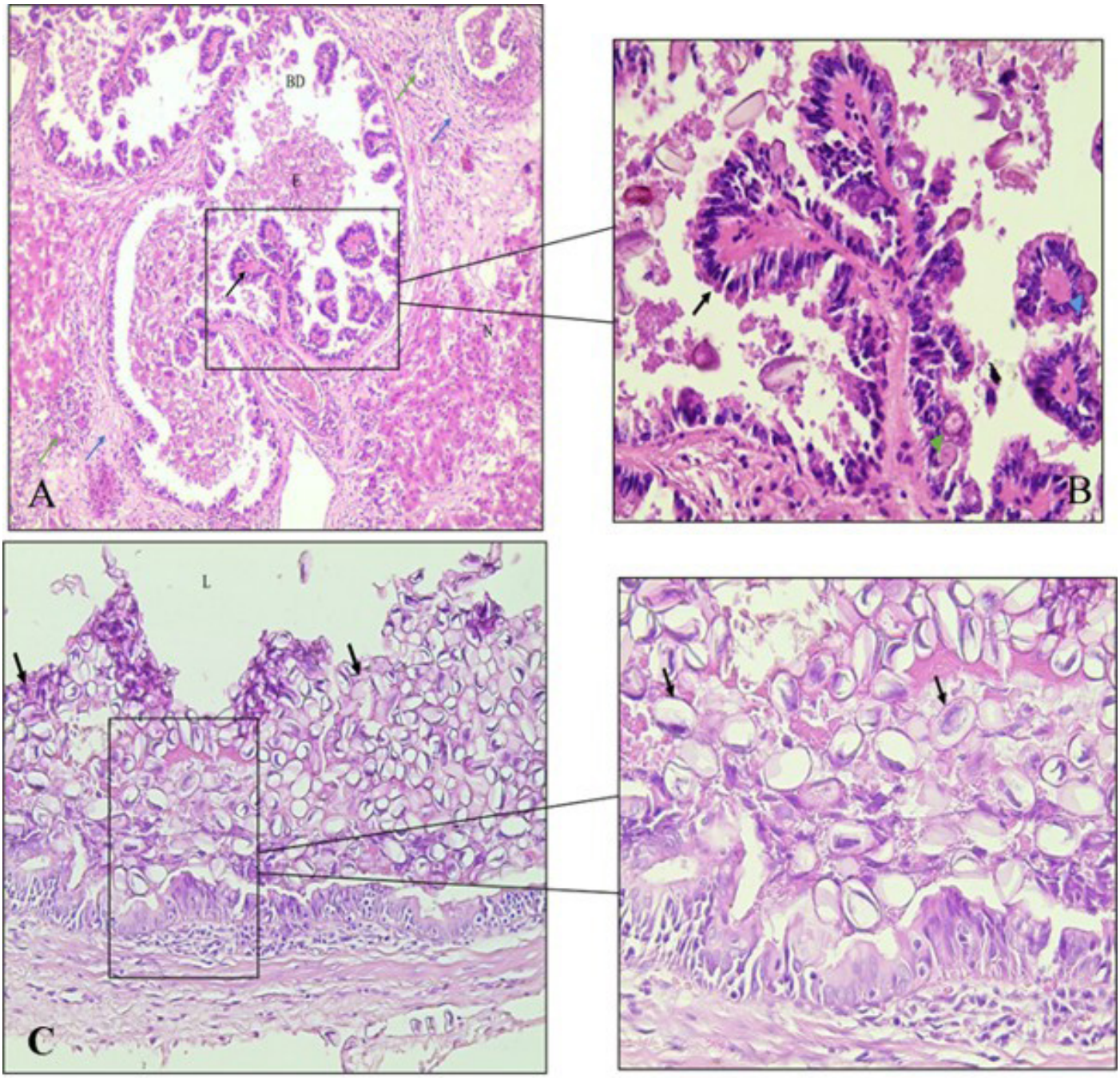

Figure 11: Histopathological section of the liver and gallbladder of the Se-NPs-treated group: (A) Liver section shows moderate enlargement of the bile duct (BD) with moderate-to-marked epithelial hyperplasia and papillary projections (black arrow). There is moderate-to-marked multifocal periductal fibrosis associated with mononuclear cell infiltration (blue arrow), newly formed bile ducts (green arrow), and moderate hepatocellular necrosis (N). (B) High magnification reveals moderate numbers of various developmental stages (E), including macrogametes (green arrowhead), microgametes (blue arrowhead), and oocysts (black arrowhead). (C) The gallbladder section shows a mildly dilated lumen (L) with flattened epithelium, containing a moderate number of oocysts (black arrows). (H and E, 10X, 40X).

A combination of antigen and Se-NPs brought the most significant histological protection in the treatment groups. The liver parenchyma and bile ducts were relatively unchanged, with slight bile duct hyperplasia, sparse oocyst occurrences, low levels of inflammatory infiltration, and a regular architecture of the gallbladder, indicating a synergistic immunoprotective effect of the complex of antigen-selenium nanoparticles. Similar hepatoprotective and lesion-reducing properties of the nano-selenium-based preparations have been described in drug coccidiosis disease and other toxin- or infection-related liver diseases (Metwally *et al*., 2022; Abdel-Gaber *et al*., 2023). Antigen provided moderate protection, with reduced but still evident bile duct proliferation, residual oocysts/gametocytes, and milder hepatocellular changes, in line with earlier work showing partial but incomplete

histological improvement with antigen immunisation, as reported by Xiao *et al*. (2022) and Deng *et al*. (2024). The treatment with diclazuril resulted in a significant regression of the hyperplasia of the bile-duct, reduced the number of oocysts, and improved the hepatic architecture, which is consistent with earlier studies that diclazuril significantly improves hepatic lesion (Allam *et al*., 2020). Selenium nanoparticles administered have provided intermediate protection: bile-duct and gallbladder lesions were reduced but not completely repaired, with moderate hyperplasia, reduced parasitic stages, and partial parenchymal recovery; this finding is in line with literature that shows that Se-NPs have natural antioxidant and tissue-protectant effects, but are less effective than antigen-targeted immunization prevention measures when used alone (Abdel-Gaber *et al*., 2023). Despite the current study supporting the therapeutic relevance of the Ag + -Se -NP complex, there are some limitations to consider. To start with, it was an experimental cohort comprising only male local rabbits of a single age, which limits the extrapolation of the results to other breeds or to females. Secondly, transient protective effects were observed, but long-term safety and the potential for tissue accretion of Se-NPs should also be fully evaluated. Further research ought thus to involve a comprehensive characterization of immune cell phenotype and the evaluation of the antigen protective ability across different strains of the *Eimeria* species.

CONCLUSIONS

To sum up, the use of green-synthesized selenium nanoparticles (Se-NPs) as an immunomodulatory vehicle of antigens of *Eimeria stiedae* was demonstrated. A composite-based formulation significantly outperforms individual therapies and traditional diagnostic methods, such as diclazuril, in reducing hepatic lesions and enhancing humoral immunity. Therefore, these findings suggest that the Ag–Se-NP construct represents a promising strategy for the future development of vaccines against rabbit coccidiosis.

ACKNOWLEDGEMENT

The authors would like to thank the College of Veterinary Medicine and the College of Science, University of Baghdad, for providing the necessary facilities and support to conduct this research.

NOVELTY STATEMENT

To the best of our knowledge, this is the first study to evaluate the synergistic immunotherapeutic impact of green-synthesized selenium nanoparticles combined with sonicated *Eimeria stiedae* antigens on hepatic coccidiosis in local rabbit breeds, particularly highlighting the modulation of Adenosine Deaminase (ADA) enzyme activity.

AUTHOR'S CONTRIBUTION

Tuqa Najim Al-Waely conducted the experimental work, data collection, laboratory analyses, data interpretation, statistical analysis, and manuscript writing. Haider Mohammed Ali Al-Rubaie and Laith Ahmed Yaaqoob supervised the study, provided scientific guidance, monitored the research progress, and critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethical approval

The experiment was conducted under ethical approval from the Animal Care and Use Committee, College of Veterinary Medicine, University of Baghdad (protocol P.G. 962/12.5.2024).

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abd El-Ghany WA (2020). Coccidiosis: A parasitic disease of significant importance in rabbits. World Vet. J., 10: 499–507. https://doi.org/10.54203/scil.2020.wvj60

Abdel-Gaber R, Al-Quraishy S, Al-Hoshani N, Al-Otaibi T, Omer SA, Alajmi F, Mohammed OB (2023). Morphological and molecular phylogenetic analyses of the apicomplexan parasites, *Eimeria media* and *Eimeria stiedai*, infecting the domestic rabbits, *Oryctolagus cuniculus*. J. King Saud Univ. Sci., 35: 102935. https://doi.org/10.1016/j.jksus.2023.102935

Abdul-Hamead AA (2020). Fabrication and AFM characterisation of selenium recycled nano particles by pulse laser evaporation and thermal evaporation. Mater. Res. Express, 6: 1250k2. https://doi.org/10.1088/2053-1591/ab6a50

Allam T, AbdelGaber M, Thabet N, AbouLaila M, Elkhatam A (2020). Clinicopathological effects of diclazuril prophylaxis and treatment on rabbits experimentally infected with *Eimeria stiedae*. Damanhour J. Vet. Sci., 4: 20–28. https://doi.org/10.21608/djvs.2020.92810

Al-Naimi RAS, Khalaf OH, Tano SY, Al-Taee EH (2012). Pathological study of hepatic coccidiosis in naturally infected rabbits. Al-Qadisiya J. Vet. Med. Sci., 11: 63–69. https://doi.org/10.29079/vol11iss1art172

Al-Taee MNK, Al-Zubaidi MTS (2017). Protection against *Eimeria stiedae* in rabbits by using sonicated sporulated oocyst vaccine. J. Entomol. Zool. Stud., 5: 579–585.

Ansari JA, Malik JA, Ahmed S, Manzoor M, Ahemad N, Anwar

S (2024). Recent advances in the therapeutic applications of selenium nanoparticles. Mol. Biol. Rep., 51: 688. https://doi.org/10.1007/s11033-024-09598-z

Asherson GL, Rose ME (1963). Autoantibody production in rabbits: III. The effect of infection with *Eimeria stiedae* and its relation to natural antibody. Immunology, 6: 207.

Ashrafi-Saiedlou S, Rasouli-Sadaghiani M, Fattahi M, Ghosta Y (2025). Biosynthesis and characterization of iron oxide nanoparticles fabricated using cell-free supernatant of *Pseudomonas fluorescens* for antibacterial, antifungal, antioxidant, and photocatalytic applications. Sci. Rep., 15: 1018. https://doi.org/10.1038/s41598-024-84974-0

Athanasiou LV, Tsokana CN, Doukas D, Kantere MC, Katsoulos PD, Papakonstantinou GI, Dedousi A (2023). Hepatic coccidiosis in wild rabbits in Greece: parasite detection on liver imprints and the associated biochemical profile. Vet. Sci., 10: 248. https://doi.org/10.3390/vetsci10040248

Awade M, Youssef M, Alakabawy L (2019). Immunisation of V-line rabbits against intestinal coccidiosis by using attenuated *Eimeria oocysts*. Benha Vet. Med. J., 36: 314–324. https://doi.org/10.21608/bvmj.2019.16352.1084

Bharti P, Tanveer S, Wani ZA, Rather SA, Parray ZA (2025). Insight into poultry coccidiosis: A dual focus on anticoccidial drugs and herbal plants for pharmacological and prevention strategies. J. Parasit. Dis., pp. 1–18. https://doi.org/10.1007/s12639-025-01805-3

Cam Y, Atasever A, Eraslan G, Kibar M, Atalay O, Beyaz L, Inci A, Liman BC (2008). Eimeria stiedae: Experimental infection in rabbits and the effect of treatment with toltrazuril and ivermectin. Exp. Parasitol., 119: 164–172. https://doi.org/10.1016/j.exppara.2008.01.005

Chen G, Yang F, Fan S, Jin H, Liao K, Li X, Pi J (2022). Immunomodulatory roles of selenium nanoparticles: Novel arts for potential immunotherapy strategy development. Front. Immunol., 13: 956181. https://doi.org/10.3389/fimmu.2022.956181

Contreras-Aguilar MD, Tvarijonaviciute A, Monkeviciene I, Martín-Cuervo M, González-Arostegui LG, Franco-Martínez L, Escribano D (2020). Characterisation of total adenosine deaminase activity (ADA) and its isoenzymes in saliva and serum in health and inflammatory conditions in four different species: An analytical and clinical validation pilot study. BMC Vet. Res., 16: 384. https://doi.org/10.1186/s12917-020-02574-2

Deng M, Hou T, Wei Y, Zeng W, Guo Y, Li N, Feng Y (2024). Transcriptional profiling of the rabbit liver infected with *Eimeria stiedae* reveals dynamic host cell responses during the induction and resolution of cholangitis. Transbound. Emerg. Dis., 2024: 4168719. https://doi.org/10.1155/2024/4168719

Hegazi AG, Shanawany EEE, El-Houssiny AS, Hassan SE, Desouky HM, El-Metenawy TM, Abdel-Rahman EH (2023). Attenuation of the pathogenesis of *Eimeria stiedae* sporulated oocysts using Egyptian alginate propolis nanoparticles. BMC Vet. Res., 19: 127. https://doi.org/10.1186/s12917-023-03689-y

Kadhim LI, Al-Zubaidi MT, Saegh HAA (2018). Influence of dietary supplementation of *Nigella sativa* on experimental coccidiosis in broiler chickens. J. Entomol. Zool. Stud., 6: 652–656.

Li H, Zhang J, Wang T, Luo W, Zhou Q, Jiang G (2008). Elemental selenium particles at nano-size (Nano-Se) are more toxic to Medaka (*Oryzias latipes*) as a consequence of hyper-accumulation of selenium: A comparison with sodium selenite. Aquat. Toxicol., 89: 251–256. https://doi.org/10.1016/j.aquatox.2008.07.008

Liu J, Zhang R, Xu ZP (2019). Nanoparticle-based nanomedicines to promote cancer immunotherapy: Recent advances and future directions. Small, 15: 1900262. https://doi.org/10.1002/smll.201900262

Metwally DM, Alkhuriji AF, Barakat IA, Baghdadi HB, El-Khadragy MF, Al-Megrin WAI, Alajmi FE (2022). Protective effect of litchi chinensis peel extract-prepared nanoparticles on rabbits experimentally infected with *Eimeria stiedae*. Animals, 12: 3098. https://doi.org/10.3390/ani12223098

Nadia MT, Kadria N, Olfat AM, Soad EH (2012). ELISA assessment in the diagnosis of hepatic coccidiosis in experimentally infected rabbits. Glob. Vet., 9: 517–523.

Puri A, Patil S (2022). Biogenic synthesis of selenium nanoparticles using *Diospyros montana* bark extract: Characterisation, antioxidant, antibacterial, and antiproliferative activity. Biosci. Biotechnol. Res. Asia, 19: 423–441. https://doi.org/10.13005/bbra/2997

Rafid K, Al-Naimi RA, Abed HH, Al-Tace EH (2015). The therapeutic effect of *Nigella sativa* L. seed oil on experimentally infected rabbits with hepatic coccidiosis. Iraqi J. Vet. Med., 39: 16–22. https://doi.org/10.30539/iraqijvm.v39i1.189

Saravanan K, Madhaiyan M, Periyasamy P, Manivannan P, Bayrakdar A, Balakrishnan V (2025). Green synthesis and detailed characterisation of selenium nanoparticles derived from *Alangium salviifolium* (Lf) Wangerin. Chem. Phys. Impact, 10: 100876. https://doi.org/10.1016/j.chphi.2025.100876

Sentkowska A, Folcik J, Szmytke J, Grudniak A (2025). Is there any correlation between green synthesis parameters and the properties of obtained selenium nanoparticles? Molecules, 30: 2865. https://doi.org/10.3390/molecules30132865

Sivajothi S, Reddy BS, Rayulu VC (2016). Study on impression smears of hepatic coccidiosis in rabbits. J. Parasit. Dis., 40: 906–909. https://doi.org/10.1007/s12639-014-0602-8

Sorour SS, Asa SA, Elhawary NM, Ghazy EW, El-Latif AA, El-Abasy MA, Khalifa HO (2018). Anticoccidial and hepatoprotective effects of artemisinin liquid extract, cinnamon essential oil, and clove essential oil against *Eimeria stiedae* infection in rabbits.

SPSS (2019). Statistical Packages of Social Sciences (SPSS)/ IBM Statistics 26: Step-by-Step 16th Edition.

Steffen KD, Carlos MJ, Carina B, Soledad TM, Omar AR, Manuel UJ, Gastón M (2025). *Eimeria stiedae* in domestic rabbits (*Oryctolagus cuniculus*) farmed in agroecological and industrial systems in Argentina. Vet. Parasitol., 59: 101234. https://doi.org/10.1016/j.vprsr.2025.101234

Sun Y, Chen D, Pan Y, Qu W, Hao H, Wang X, Xie S (2019). Nanoparticles for antiparasitic drug delivery. Drug Deliv., 26: 1206–1221. https://doi.org/10.1080/10717544.2019.1692968

Tadayon S, Razavi SM, Nazifi S (2016). Dynamic patterns of systemic innate immunity and inflammation-associated factors in experimental caprine coccidiosis. Korean J. Parasitol., 54: 719. https://doi.org/10.3347/kjp.2016.54.6.719

Talab MA, Badr FI (2026). Immunological, biochemical, and histological evaluation of sonicated *Theileria annulata* antigens and methionine in rabbit models. Iran. J. Vet. Med.,

20: 99–112. https://doi.org/10.32598/ijvm.20.1.1005770

Tonin AA, Da Silva AS, Ruchel JB, Rezer JF, Camillo G, Faccio L, Lopes ST (2013). E-NTPDase and E-ADA activities in lymphocytes associated with the immune response of rats experimentally infected with *Toxoplasma gondii*. Exp. Parasitol., 135: 325–330. https://doi.org/10.1016/j.exppara.2013.07.014

Vekariya KK, Kaur J, Tikoo K (2012). ERα signalling imparts chemotherapeutic selectivity to selenium nanoparticles in breast cancer. Nanomed. Nanotechnol. Biol. Med., 8: 1125–1132. https://doi.org/10.1016/j.nano.2011.12.003

Xiao J, Zheng R, Bai X, Pu J, Chen H, Gu X, Yang G (2022). Preliminary evaluation of the protective effects of recombinant AMA1 and IMP1 against *Eimeria stiedae* infection in rabbits. Parasit. Vectors, 15: 400. https://doi.org/10.1186/s13071-022-05492-4

Yaaqoob LA, Abed RM, Kamona ZK, Altaee MF (2022). Evaluate the ability of titanium oxide nanoparticles to increase the production of prodigiosin and phenasine from *Serratia marcescens* and *Pseudomonas aeruginosa,* respectively. Iraqi J. Agric. Sci., 53: 496–504. https://doi.org/10.36103/ijas.v53i3.1557

Yarim GF, Yagci BB, Ertekin A, Kazak F (2016). Decreased serum adenosine deaminase activity correlated with clinical score and serum proteins in calves with cryptosporidiosis. Pak. J. Zool., 48.