Original Article

Immunological, Biochemical, and Histological Evaluation of Sonicated *Theileria annulata* Antigens and Methionine in Rabbit Models

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ABSTRACT

Background: *Theileria annulata* is a hemoprotozoan parasite transmitted by ticks, responsible for tropical theileriosis, an important disease in cattle that leads to high mortality and morbidity rates. The disease causes economic losses to the animals due to reduced productivity, treatment costs, and death, especially in endemic areas.

Objectives: This study examined rabbit immunization with sonicated *T. annulata* antigen, determined the effects of administering immunomodulatory L-methionine, and performed the immunological, biochemical, and histopathological analysis to discover strategies to control the parasite's spread.

Methods: Forty-eight rabbits were divided into four groups, each comprising 12 rabbits. The first group (control) administered an injection of distilled water and functioned as the control group. The second group (antigen) was inoculated intraperitoneally with 500 µg of purified sonicated *T. annulata* piroplasm antigen. The third group (antigen + L-methionine) was inoculated similarly to the second group and received supplementation with L-methionine. The fourth group administered L-methionine. The immunological analysis of the rabbit groups was performed on day 21 of the trial. The investigation included evaluations of immune response parameters (IFN-γ, IL-10, TGF-β1, and IgG), biochemical serum analysis (malondialdehyde [MDA] and total antioxidant capacity [TAC]), and histopathological analysis of the mesenteric lymph nodes.

Results: Immunization significantly elevated IFN-γ, IL-10, TGF-β1, and IgG levels compared to controls. Immunization combined with L-methionine (G3) resulted in the highest levels, indicating synergy. However, L-methionine alone (G4) raised these values to a lesser extent. Group G4 (L-methionine) improved antioxidant capability, but group G2 (immunized) exhibited higher MDA levels, indicating oxidative stress. Histologically, group G2 showed follicular hyperplasia and hemorrhages; group G3 exhibited mixed immune cell infiltration and minor vascular congestion; the group G4 displayed mild apoptotic alterations and enhanced cortical cellularity. L-methionine enhanced immunological and antioxidant responses, especially when combined with vaccination.

Conclusion: Immunization significantly enhances immune responses, while L-methionine appears to further amplify these effects when combined with immunization. L-methionine alone also contributes to immune regulation, but to a lesser extent.

Keywords: Antioxidant potential, Immunization, Methionine, TGF-β1, Tropical theileriosis.

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Introduction

Theileria annulata causes tropical theileriosis and is transmitted by ticks, specifically the *Hyalomma* species. In endemic areas, they pose significant economic challenges The clinical signs of theileriosis in cattle are elevated body temperature, anorexia, swelling of superficial lymph nodes, weight loss, drop in milk yield, nasal discharge, lacrimation, dyspnea, anemia, and in later stages, sometimes diarrhea and dysentery, often accompanied by the presence of ticks on the body (Abdel-Rady et al., 2023; AL-ani & Yousif, 2023). Iraq is among several countries affected by tropical theileriosis, which poses a significant problem. Epidemiological studies have revealed that the disease is common in various provinces, including Kurdistan, Mosul, Diyala, Baghdad, Basra, and Fallujah (Alsaadi & Faraj, 2020; Salih et al., 2024).

The *Theileria* vaccines offer an eco-friendly solution, but they are currently unavailable for most of these diseases. Several reasons contribute to the current short supply of vaccines: some diseases go unnoticed, and insufficient funding is allocated for research; the biology of the majority of these parasites, their interactions with hosts, and the immune systems that provide protection are thus all areas of significant unknown. Additionally, the complexity of parasite-host interactions, along with our inability to cultivate and genetically modify certain parasites and to fully understand the immunological systems underlying protection, typically makes vaccine development challenging (Kawan, 2019; Florin-Christensen et al., 2021).

Immunization is a highly effective strategy for preventing infectious diseases, and vaccines for bovine theileriosis are among the limited options available for animal protozoal diseases (Agina et al., 2020). Preventive vaccines are the best environmentally friendly option (Florin-Christensen et al., 2014). Vaccines for *T. annulata* are categorized into three primary types: Live attenuated, infection and treatment method, and Subunit vaccines (Nene & Morrison, 2016).

Subunit vaccines are defined as vaccine agents that consist of one or more components of a pathogen instead of the complete pathogen. Subunit vaccines consist of one or more recombinant peptides, proteins, or polysaccharides typically found in the structure of the target pathogen (Dudek et al., 2010).

Subunit vaccinations are considered to be safer and more cost-effective (Kallerup & Foged, 2015) and are thought to offer varying levels of protection (Morrison & McKeever, 2006).

Methionine, an essential amino acid in the diet, has significant nutritional value and crucial physiological effects, including growth stimulation and antioxidant capability (Liu et al., 2019; Magnuson et al., 2020), detoxification, antitumor and anticancer properties, resistance to coccidium infection, methyl transfer, and protein synthesis (Teng et al., 2023). It has a close relationship with the immune function, which affects both the organism's specific and nonspecific immune function, as well as the growth and development of immune organs (Elmada et al., 2016). Additionally, methionine is an essential precursor to taurine and glutathione, two intracellular antioxidants (Brosnan & Brosnan, 2006). Antioxidant imbalance may promote inflammation in the body (Sordillo & Aitken, 2009). Substances originating from plants have been formulated as an alternative strategy for managing parasite diseases; these substances not only attack parasites but may also provide protective advantages to the organs of affected hosts (Abu Hawsah et al., 2023).

This study aimed to evaluate the immune response induced by sonicated *T. annulata* antigens in rabbits. It also assessed the effect of L-methionine as an immunomodulatory supplement, both alone and in combination with the antigen. Biochemical parameters related to oxidative stress and antioxidant capacity were measured to understand systemic responses. Additionally, histopathological changes in mesenteric lymph nodes were examined to reveal tissue-level immune activation.

Materials and Methods

Experimental design

Forty-eight healthy adult rabbits (*Oryctolagus cuniculus*), aged 8 to 9 weeks, were utilized for this investigation from January to May 2024. The rabbits were categorized into four groups, each comprising 12 rabbits. The initial group received an injection of distilled water and served as the control group. The second group was immunized intraperitoneally with 500 µg of sonicated *T. annulata* antigen. The third group was immunized as the second group and supplemented with L-methionine. The fourth group was administered L-methionine. The immunological study of the rabbit groups was conducted on day 21 of the experiment.

Blood samples

Blood samples were taken on day 21st post-immunization for serum extraction. The serum was obtained by centrifuging 2 mL of blood at 3000 rpm for 15 minutes, and was stored at -80 ˚C until the enzyme-linked immunosorbent assay (ELISA) (Razmi et al., 2019; Özdek et al., 2020).

Blood smear analysis

Thin blood smears were obtained and stained with a 5% Giemsa solution for 30 to 60 minutes. The smears were then examined under a light microscope to evaluate the morphological features of *Theileria* parasites in cattle (Sabbar, 2016; Faraj et al., 2019).

Preparation of sonicated *T. annulata* antigens

Blood infected with *T. annulata* (2.69×10^6), exhibiting a piroplasm parasitemia of 28% was collected in a heparinized vacutainer. The sonicated *T. annulata* antigen was generated from purified *T. annulata*. Cells were disrupted with a cell ultrasonicator deflector (Sonifier-Branson 250, Branson, USA) (Boulter & Hall, 1999; Schmuckli-Maurer et al., 2008). The protein concentration of the final supernatant was then determined using the Roche Cobas c311 assays (Switzerland) (Bradford, 1976).

Immunization dose

Primary immunization was conducted using 500 µg of purified sonicated *T. annulata* piroplasm antigen, administered intraperitoneally. Booster doses of 500 µg of the same antigen were administered two weeks after the primary immunization, via intraperitoneal injection (Lastuti et al., 2018; Greenfield, 2020).

L-methionine preparation

L-methionine (France), used as an immunomodulator at a dosage of 8.3 g/kg/day for one month when administered orally (Oloruntola, 2020), is prepared from a stock solution that is stored at 5 ˚C in a dry place away from light. This solution has a concentration of 880 mg/mL and is administered at a dose volume of 9.43 mL for every kg of animal body weight (Consortium, 2016) using a stainless steel gavage needle.

Immunological analysis

For immunological analysis, blood samples were collected on the 21st day post-immunization. After serum separation, the concentrations of interferon-gamma (IFN-γ, pg/mL), interleukin-10 (IL-10, pg/mL), and transforming growth factor-beta 1 (TGF-β1, ng/mL) were measured using commercial sandwich ELISA kits, while immunoglobulin G (IgG, µg/mL) levels were assessed using an indirect ELISA kit (Elabscience, USA), following the manufacturer's instructions.

Briefly, for cytokines (IFN-γ, IL-10, and TGF-β1), 100 µL of diluted standard, blank, or serum sample was added to designated wells of a 96-well microplate and incubated at 37 ˚C for 90 minutes. All measurements were performed in duplicate. After decanting the wells (without washing), 100 µL of a biotinylated detection antibody working solution was added. The plates were sealed and incubated for 1 hour at 37 ˚C. The wells were then washed three times using 350 µL of wash buffer per well (with 1-minute soaking), followed by gentle drying on absorbent paper. Then, 100 µL of horseradish peroxidase (HRP)-conjugated working solution was added to each well, and the mixture was incubated for 30 minutes at 37 ˚C. After five additional wash cycles, 90 µL of substrate reagent was added, and the plates were incubated in the dark at 37 ˚C for approximately 15 minutes. The reaction was stopped by adding 50 µL of a stop solution, and optical density (OD) was measured immediately at 450 nm using a microplate reader.

For IgG detection, five wells were used for standard dilutions, one for blank samples, and the remaining for serum samples. Each well received 50 µL of standard, blank, or sample, followed by 50 µL of detection reagent A. The plate was gently shaken, sealed, and incubated at 37 ˚C for 1 hour. After aspiration, the wells were washed five times with 350 µL of wash buffer, allowing for a 1-2 minute soak per cycle. Then, 90 µL of substrate solution was added, and the plate was incubated in the dark at 37 ˚C for 10–20 minutes. The reaction was stopped with 50 µL of a stop solution, and the OD was measured at 450 nm.

Biochemical serum analysis

The evaluation of oxidative stress biomarkers, including malondialdehyde (MDA, ng/mL) and total antioxidant capacity (TAC, U/mL), was conducted according to the manufacturer's instructions (Elabscience, USA). MDA was quantified via a competitive ELISA kit, while TAC was assessed using a colorimetric assay. Blood samples were collected on days 14 and 21 post-infection, and serum samples were separated for analysis. For MDA determination, 50 µL of diluted standard, blank, or sample was added to each well, followed immediately by 50 µL of biotinylated detection antibody solution. The

plates were sealed and incubated at 37 ˚C for 45 minutes. After incubation, the wells were washed three times with 350 µL of wash buffer for each well, and then incubated with 100 µL of HRP-conjugated working solution for 30 minutes at 37 ˚C, followed by five additional washes. Subsequently, 90 µL of substrate reagent was added, and the mixture was incubated in the dark at 37 ˚C for 15 minutes. The reaction was stopped by adding 50 µL of a stop solution. OD was measured at 450 nm.

TAC levels were measured using a colorimetric method. In both the sample and control tubes (5-mL Eppendorf tubes), 1 mL of buffer solution was added. To the sample tube, 0.1 mL of serum was added, whereas the control remained without serum. Then, 2.0 mL of chromogenic working solution and 0.5 mL of ferric salt working solution were added to both tubes. After thorough mixing, the tubes were incubated at 37 ˚C for 30 minutes. Subsequently, 0.1 mL of a stop solution was added to each tube. Then, 0.1 mL of serum was added to the control tube. The mixtures were allowed to stand for 10 minutes at room temperature. Absorbance was measured at 520 nm using a 1-cm quartz cuvette, with double-distilled water used for zeroing the spectrophotometer.

Histopathological examination

Histological analysis was performed on all experimental animals after their sacrificed on day 21 post-immunization. Samples were obtained from the mesenteric lymph node. We preserved the tissues in a 10% formaldehyde solution and subsequently processed them as per standard protocol. Paraffin blocks containing cultured tissue slices were sectioned, stained with hematoxylin and eosin (H&E), and subsequently analyzed using light microscopy (Bancroft et al., 2008).

Statistical analysis

SPSS software, version 25 was utilized to identify the effect of various factors on study parameters. A statistically significant difference, determined using Fisher's least significant difference (LSD) test, was employed to compare means significantly (ANOVA, both two-way and one-way) with probabilities of 0.05 and 0.01 in this investigation.

Results

Immunization alone resulted in a significant elevation in IFN-γ levels compared to the control group. The combination of immunization and L-methionine (G3) produced the highest levels of IFN-γ, suggesting a synergistic effect. L-methionine alone (G4) caused a moderate increase, though lower than the immunized groups (Table 1 and Figure 1). Immunized groups (G2 and G3) demonstrated significantly elevated IL-10 levels compared to the control group. L-methionine alone (G4) increased IL-10, although to lower levels than the immunized groups. Immunization increased IL-10 production, with L-methionine potentially enhancing this response (Table 1 and Figure 2). The highest levels of TGF-β1, a regulatory cytokine, were observed in the immunization and L-methionine group (G3). Immunization alone (G2) and L-methionine ingestion (G4) resulted in elevated TGF-β1 levels, even to different levels. Immunization and L-methionine played roles in immune regulation (Table 1 and Figure 3). The immunization alone led to a significant increase in IgG levels, whereas the combination of immunization and L-methionine (G3) elicited the highest IgG response. L-methionine alone (G4) resulted in a moderate increase, yet it was lower than the immunized groups. This suggests that L-methionine supplementation may enhance antibody production when used in conjunction with immunization (Table 1 and Figure 4).

MDA levels were studied 21 days post-immunization (Table 2 and Figure 5). The control negative group (G1) exhibited an MDA level of 121.84±4.07, which serves as a baseline. The vaccinated group (G2) exhibited the highest MDA levels (127.83±10.71 ng/mL), signifying oxidative damage resulting from immunological activation. The mixed group (G3), which was administered both vaccination and methionine, exhibited the lowest MDA levels (106.81±3.06 ng/mL), indicating the effectiveness of methionine in mitigating oxidative stress. The methionine-administered group (G4) exhibited an intermediate MDA level (117.92±4.32 ng/mL), suggesting that methionine alone has certain antioxidant properties, but is less effective than in the combined group. These findings underscore the potential function of methionine in alleviating oxidative damage caused by vaccination.

The effect of different treatments on TAC values in rabbits was studied 21 days after immunization (Table 2 and Figure 6). The G1 exhibited a TAC value of 5.09±0.12 U/mL, indicating the baseline antioxidant status. The immunized group (G2) exhibited a comparable TAC value (5.05±0.32 U/mL), suggesting that immunization alone did not enhance antioxidant defenses. The G3 (immunized and methionine-treated) demonstrated the highest TAC values (11.09±0.35 U/mL), indicating that methionine supplementation substantially enhanced antioxidant capability following immunization. The G4 had an increased TAC value (10.21±0.09), suggesting that methionine alone increases antioxidant defense, although

Table 1. Effect of rabbit groups and time on IFN-γ, IL-10, TGF-β1, and IgG levels

Groups	Mean±SE			
	21 Days Post-immunization			
	IFN-γ (pg/mL)	IL-10 (pg/mL)	TGF-β1 (ng/mL)	IgG (µg/mL)
G1	212.04±9.02^{D}	40.29±1.73^{C}	1.237±0.15^{C}	40.43±3.71^{D}
G2	460.09±13.81^{B}	69.86±1.52^{A}	4.175±0.34^{AB}	200.70±3.91^{B}
G3	505.91±4.50^{A}	73.17±3.20^{A}	5.218±0.04^{A}	239.55±12.14^{A}
G4	395.02±7.57^{C}	61.58±1.62^{B}	3.712±0.22^{B}	111.34±2.73^{C}
LSD (P)	36.739 (0.0001)**	6.028 (0.0001)*	1.407 (0.0017)**	28.552 (0.0001)**

**P≤0.01.

Note: Different letters in the same column represent significant differences. LSD: Fisher's least significant difference test. Degree of freedom: 4-1=3.

it is more effective when used in conjunction with vaccination. The data indicate that methionine is essential for augmenting antioxidant capability, especially in vaccinated rabbits.

Histopathological alterations

All animals were thoroughly examined grossly after sacrifice, and no gross lesions were observed in any of the groups. The mesenteric lymph nodes from all rabbits were collected and analyzed histologically to ensure consistency. The histopathological analysis of the mesenteric lymph nodes at 21 days revealed distinct differences among the four groups. The control (G1) group exhibited no significant pathological changes, with only minimal hemorrhages observed (Figure 7). In group G2 (immunized), moderate follicular hyperplasia was noted, along with increased lymphatic cellularity and peripheral hemorrhages (Figure 8). Group G3 (immunized and methionine-treated) presented multifocal infiltration of mixed mononuclear cells (MNCs) and neutrophils, along with mild vascular congestion and pronounced follicular hyperplasia (Figure 9). In contrast, group G4 (methionine) showed mild apoptotic changes in germinal centers, accompanied by increased cellularity in the cortical layer (Figure 10). These findings suggest that immunization and methionine supplementation influence lymph node histopathology, with varying degrees of immune response and cellular activity.

Table 2. Effect of rabbit groups and time on MDA levels and TAC values

Groups	Mean±SE	
	21 Days Post-immunization	
	MDA (ng/mL)	TAC (U/mL)
G1	121.84±4.07^{AB}	5.09±0.12^{B}
G2	127.83±10.71^{A}	5.05±0.32^{B}
G3	106.81±3.06^{C}	11.09±0.35^{A}
G4	117.92±4.32^{B}	10.21±0.09^{A}
LSD (P)	9.048(0.0382)*	1.882 (0.0002)**

*P≤0.05, **P≤0.01.

Note: Different letters in the same column represent significant differences. LSD: Fisher's least significant difference test. Degree of freedom: 4-1=3.

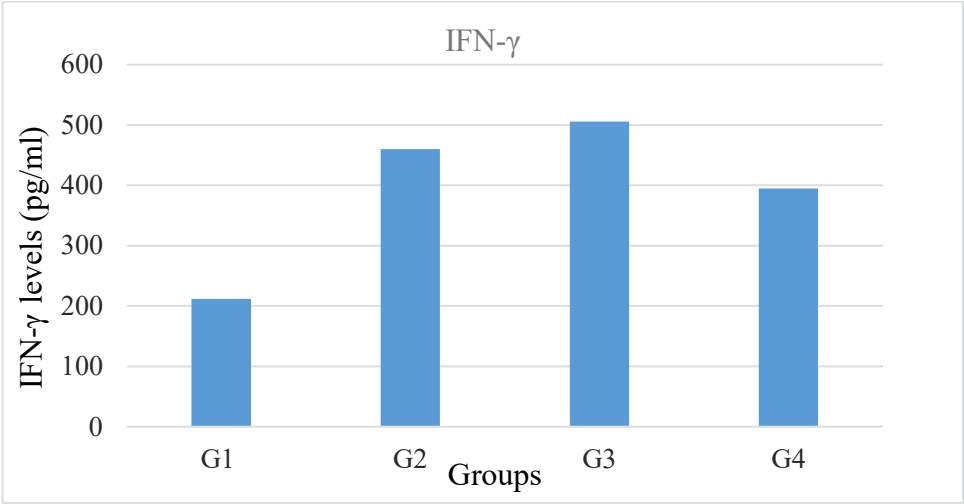

Figure 1. Serum levels of IFN-γ (pg/mL) in rabbit groups at 21 days post-immunization

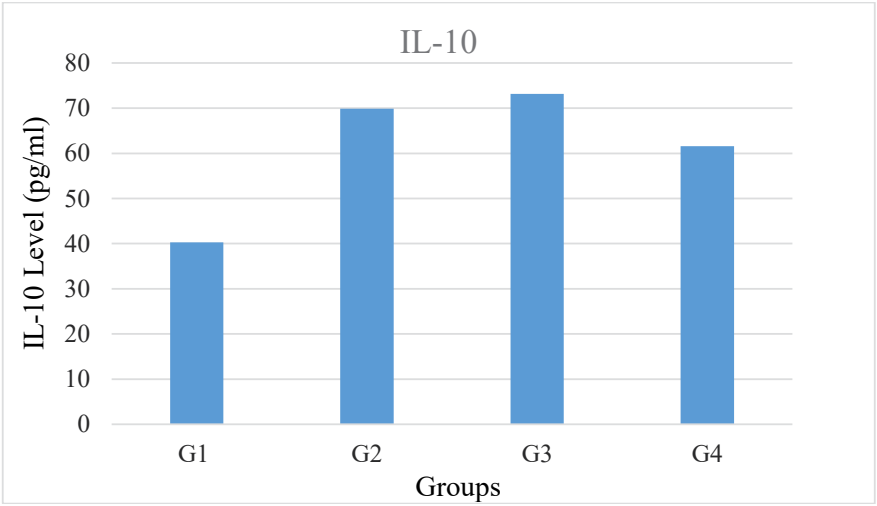

Figure 2. Serum levels of IL-10 (pg/mL) in rabbit groups at 21 days post-immunization

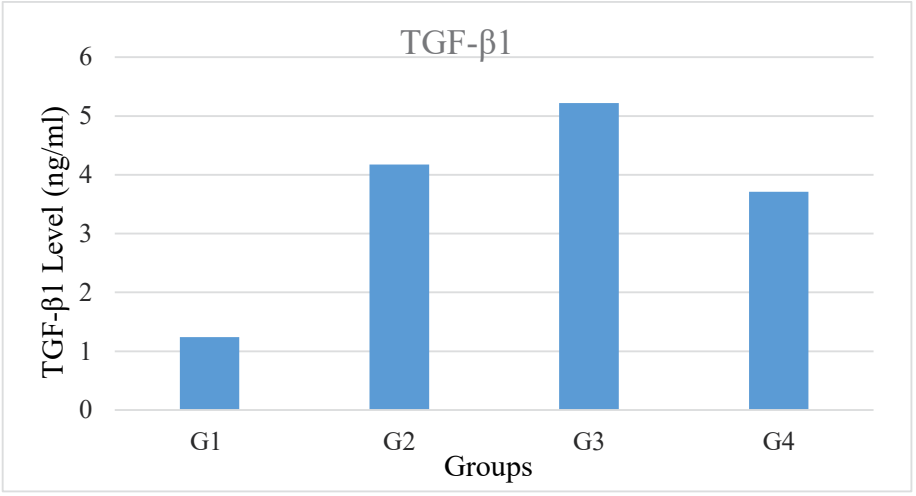

Figure 3. Serum levels of TGF-β1 (ng/mL) in rabbit groups at 21 days post-immunization

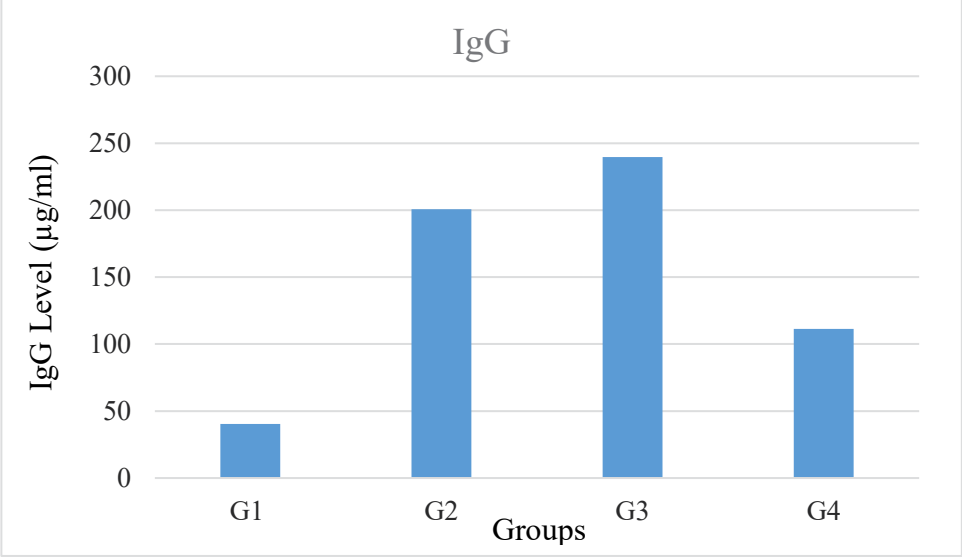

Figure 4. Serum levels of IgG (µg/mL) in rabbit groups at 21 days post-immunization

Immunized groups (G2 and G3) showed a consistent increase in all immune markers compared to the control group. L-methionine alone (G4) increased immune markers but not as strongly as immunization, indicating its supportive role in immune modulation. The histopathology findings indicated varied responses in mesenteric lymph nodes across the four groups. In group G2 (immunized), there was significant follicular hyperplasia and heightened lymphatic cellularity, characteristics of an active immunological response involving B cell activation and germinal center development in reaction to the antigen. In group G3 (immunized and methionine-treated), the presence of neutrophil infiltration, vascular congestion, and enhanced follicular hyperplasia suggests that methionine increases inflammation, possibly by influencing immune cell transport or cytokine synthesis. On the other hand, group G4 (methionine alone) exhibited minor apoptotic alterations in the germinal centers and heightened cellularity in the cortical layer, indicating a minimal inflammatory response induced by methionine. The apoptotic alterations in group G4 may indicate methionine's involvement in slowing immune cell viability and turnover. The combination of vaccination and methionine (G3) resulted in a more powerful and prominent immunological response, whereas methionine alone (G4) modulated lymph node structure.

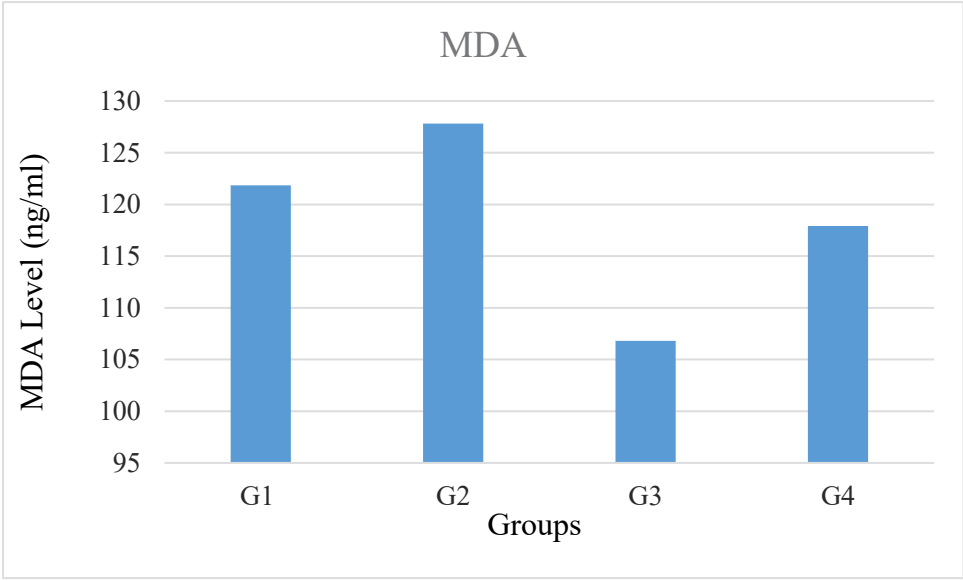

Figure 5. Serum levels of MDA (ng/mL) in rabbit groups at 21 days post-immunization

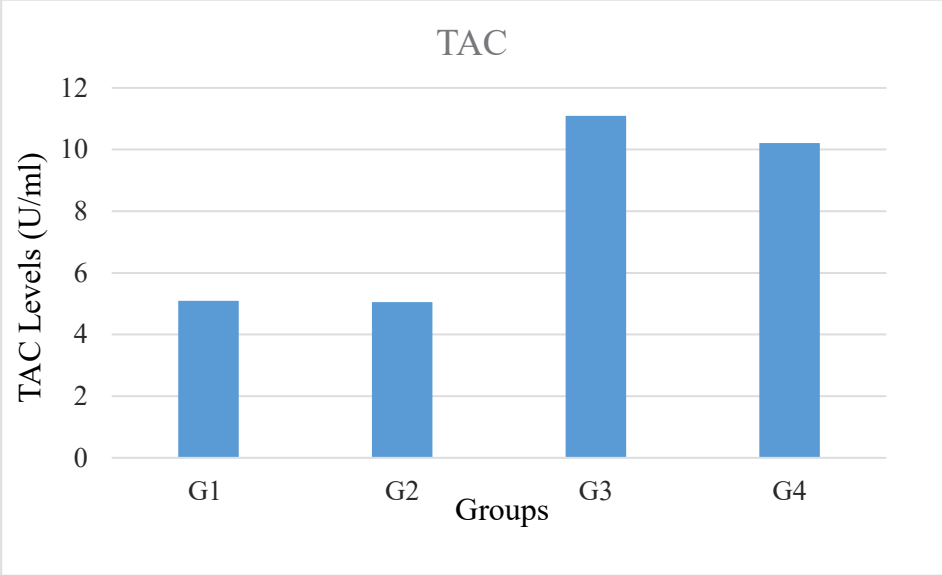

Figure 6. Serum levels of TAC (U/mL) in rabbit groups at 21 days post-immunization

Discussion

The study highlights the immunogenic potential of *Theileria sergenti* merozoite sonication in cattle, utilizing a small amount of purified and dissolved antigens for vaccination. Vaccinated animals typically exhibited hematological and biochemical profiles within normal ranges, which is a significant advancement in the development of standard *T. sergenti* vaccines (Baek et al., 1992). Duffus and Wagner successfully detected the production of *T. parva* piroplasm antibodies after immunization with purified sonicated *T. parva* antigen (Duffus & Wagner, 1974).

The subunit vaccines, particularly, are highly immunogenic and capable of inducing strong antibody responses in both mice and cattle. These vaccines, when formulated with suitable adjuvants (saponin-based or water-in-oil emulsion), elicited high titers of neutralizing antibodies that significantly inhibited *T. parva* sporozoite infectiv ity in vitro. In efficacy trials, these vaccines provided substantial protection against East Coast fever (ECF) in cattle, showing the highest level of protection (85% with water-in-oil emulsion and 77% with saponin-based adjuvant). Importantly, the vaccines achieved this protection with significantly lower doses

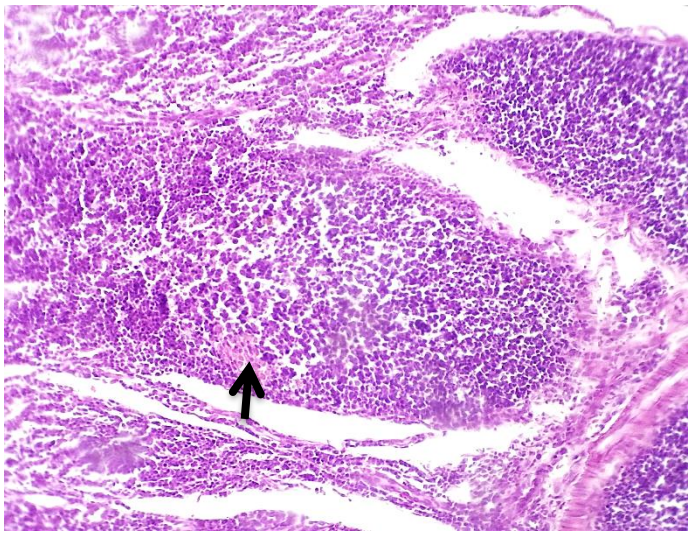

Figure 7. The histopathological section of the mesenteric lymph node in group G1 at 21 days exhibited no clear pathological alteration, with minimal hemorrhages (black arrow) (H&E stain, magnification: ×10).

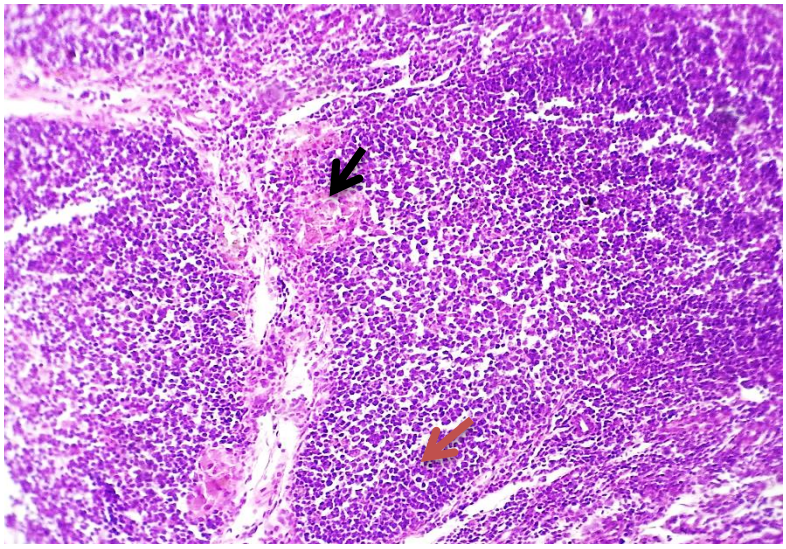

Figure 8. The histopathological section of the mesenteric lymph node in group G2 at 21 days presented moderate follicular hyperplasia, with increased lymphatic cellularity (red arrow), accompanied by peripheral hemorrhages (black arrow) (H&E stain, magnification: ×10).

of antigen (25-50 µg per inoculation) and fewer inoculations (only two) compared to previous studies, which required higher doses and multiple boosts (Kaba, 2003). The development of subunit vaccines against *Theileria orientalis* leads to effective immunization strategies that reduce parasitemia and clinical symptoms in infected cattle (Kim et al., 2004).

Immunization techniques frequently aim to trigger strong cellular and humoral immune responses. Vaccines have demonstrated efficacy in eliciting protective immunity, especially through the activation of CD8+ T cells and the secretion of cytokines, such as IFN-γ, essential for controlling intracellular parasites (Florin-Christensen et al., 2021).

The *T. annulata* vaccine elicited both humoral and cellular immune responses in animal models. Immunized rabbits and calves showed significantly higher antibody titers and increased CD4+ and CD8+ T-cell counts compared to controls, indicating a robust immune response. The study highlights the efficacy of immunoinformatics in vaccine design and creates a way for similar strategies against other parasitic diseases (Agina et al., 2020; Abid et al., 2024).

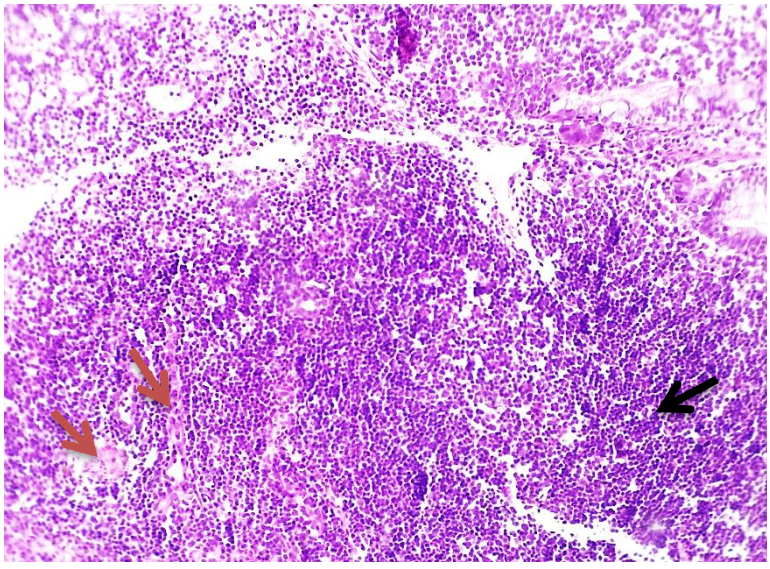

Figure 9. The histopathological section of the mesenteric lymph node in group G3 at 21 days showed mixed infiltration of MNCs and neutrophils, with mild vascular congestion (red arrow) associated with obvious follicular hyperplasia (H&E stain, magnification: ×10).

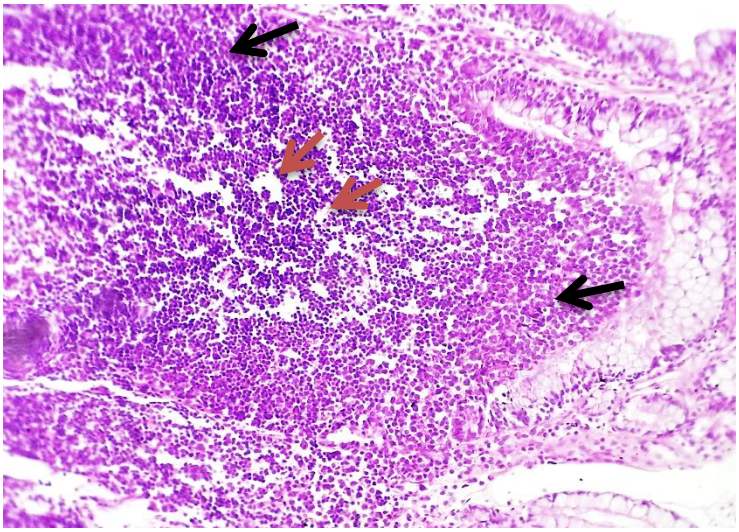

Figure 10. The histopathological section of mesenteric lymph node in group G4 at 21 days revealed mild apoptotic findings in the germinal centers (red arrow), accompanied by increased cellularity in the cortical layer (black arrow) (H&E stain, magnification: ×10)

Group G3 (immunized and methionine-treated) exhibited the highest levels in most markers. This suggests that L-methionine enhances immune responses when combined with immunization.

Research on methionine has confirmed its antioxidant, immune-boosting, and health-promoting properties in rabbits (Oloruntola, 2020). Our findings agreed with those of Grant et al., who noted that methionine supplementation reduces inflammation at the physiological level in cattle (Grant et al., 2022). Dey et al. also discovered that pretreatment with methionine eliminated pathogenic alterations, resulting in lower levels of oxidative stress and TNF-α. Additionally, methionine protected the mitochondria of obese mice given pyrazole from oxidative damage (Dey et al., 2007). Methionine deficiency induces pathological and structural alterations in the thymus gland, diminishes T cell count, serum IL-2 levels, and T cell proliferation, promotes necrotic cell accumulation, and ultimately impairs cellular immune function in broiler chickens (WU et al., 2012). Methionine demonstrated protective effects against lead-induced hematological changes when administered alone. It showed antioxidant properties, likely due to its sulfur moiety, which can scavenge free radicals (Alqayim & Asis, 2013). The role of methionine as a sulfur-containing amino acid with antioxidant properties contributes to the overall efficacy of the extract in combating oxidative stress and parasitic infections (Abu Hawsah et al., 2023).

Methionine is an essential amino acid with notable antioxidant properties that contribute to immune support and cellular protection. Research shows that methionine supplementation can reduce oxidative stress-related damage, such as gentamicin-induced nephrotoxicity in rats. These benefits highlight methionine's potential as a protective dietary additive, particularly in stress- or disease-challenged animals (Derakhshanfar et al., 2009). Methionine metabolism plays a critical role in various cellular functions, including methylation reactions, redox maintenance, and nucleotide synthesis (Sanderson et al., 2019).

Methionine, a sulfur-containing essential amino acid, demonstrated significant antioxidant properties by reducing reactive oxygen species (ROS) levels and maintaining mitochondrial functionality. It was shown to preserve mitochondrial membrane potential and prevent mitochondrial fragmentation. These findings suggest that an L-methionine-enriched diet could be beneficial in protecting neurons from oxidative imbalance and mitochondrial dysfunction, potentially slowing the progression of neurodegenerative diseases, like Parkinson's disease. Further research is necessary to explore the therapeutic potential of methionine in clinical settings (Catanesi et al., 2021).

Conclusion

Immunization with sonicated *T. annulata* antigens significantly enhances immune responses, as indicated by elevated levels of IFN-γ, IL-10, TGF-β1, and IgG. The

combination of immunization and methionine produced the strongest immune response, suggesting a synergistic effect between the antigen and the immunomodulator. Although methionine alone contributed to immune regulation and antioxidant activity, its effects were less pronounced than those of antigen immunization. Histopathological findings support this synergy, with group G3 showing marked follicular hyperplasia and inflammatory cell infiltration.

These results confirm the strong immunogenic potential of sonicated *T. annulata* antigens in rabbit models, using small amounts of purified antigen. Compared to other vaccine strategies, the use of sonicated antigens presents a promising and efficient approach for stimulating cellular and humoral immunity, aligning with previous findings on antigen-based immunization in protozoan infections. Despite the promising outcomes, further exploration of different adjuvants or delivery systems may enhance the immunogenic performance of the antigen.

Ethical Considerations

Compliance with ethical guidelines

Approval for this study was obtained from the Ethics Committee of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq (Code: 118/PG on 20/1/2025).

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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