Original Article

Pulmonary Hydatid Infection (*Echinococcus granulosus* larvae) as a Possible Inducer of Alveolar Adenocarcinoma and Immune Cell Response in Ovine Lung Tissue

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ABSTRACT

Background: Pulmonary hydatidosis infection, caused by *Echinococcus granulosus* larvae, is a zoonotic disease that stimulates remarkable histological and immunological alterations in infected host tissues.

Objectives: This study investigated the link between hydatid infection and alveolar adenocarcinoma in sheep lungs, alongside the localized immune response, including CD4+ and CD8+ T cells and PD-L1 expression.

Methods: A total of 24 hydatid-infected and 10 non-infected ovine lung samples were examined using histopathological (H&E, PAS, Masson's trichrome) and immunohistological (CD4, CD8, and PD-L1) techniques.

Results: Results demonstrated that 36% of infected lungs revealed adenocarcinoma, accompanied by notable inflammation, necrosis, and fibrosis. Immunohistological tests demonstrated an increase in CD4+ and CD8+ T-cell infiltration in infected tissues, with PD-L1 expression significantly higher in adenocarcinoma-associated samples compared to non-infected and hydatid-only lungs.

Conclusion: These results suggest that chronic hydatid infection may contribute to oncogenic transformation through modulation of immune response, particularly via exhaustion of PD-L1-mediated T-cells. This study also highlights the possible role of *E. granulosus* in pulmonary carcinogenesis and emphasizes the importance role of immune checkpoint molecules in parasitic and neoplastic evasion mechanisms.

Keywords: Pulmonary hydatidosis, CD4, CD8, PD-L1

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Introduction

Pulmonary hydatid cysts, a parasitic zoonotic infection resulting from infection with the metacestode larva stage of *Echinococcus granulosus*, represent a significant disease affecting the liver and lungs, with the lung being the second most common target after the liver (Abolhasani et al., 2024; Hashemi & Salari, 2025; Shirazi et al., 2025). The presence of hydatid cysts in the lung can induce marked histological and immunohistological alterations (depending on the size, number, and location of cysts). These include cyst encapsulation by laminated and fibrous membranes, infiltration of various inflammatory cells such as eosinophils, lymphocytes, and mast cells, as well as tissue damage characterized by pneumonia, necrosis, fibrosis, atelectasis, and emphysema (Al-Sabawi & Sadoon, 2023). Immunohistological studies reveal a prominent T-cell-mediated immune response, predominately involving CD3+ lymphocytes (a surface protein complex on T-cell co-receptor that mediates activation of both cytotoxic T cells (CD8+ naive T cells) and T helper cells (CD4+ naive T cells), indicating the host's cell-mediated immunity against the parasite (De Biase et al., 2023; Hamad et al., 2024). CD4+ (a surface glycoprotein on many cells, such as T helper cells, monocytes, and macrophages, serving as a co-receptor for the T-cell receptor) and CD8+ T cells (a surface glycoprotein of cytotoxic T lymphocytes) play vital roles in immunity against hydatid cyst infection in the lung. CD4+ T cells, fundamentally helper T cells, help regulate and orchestrate the immune response by stimulating other immune cells, including CD8+ cytotoxic T cells, which directly target infected or abnormal cells. Studies show that both CD4+ and CD8+ T cells infiltrate the tissue around hydatid cysts and are implicated in the local inflammatory response, with their presence correlating positively with granzyme B (released by cytotoxic lymphocytes) expression, a marker of cytotoxic activity (Cheraghipour et al., 2024; Hamad et al., 2024). This suggests that these T cells contribute to controlling cyst fertility by enhancing inflammation and cytotoxic effects, potentially limiting parasite survival. These pathological and immune changes disrupt normal lung structure and function, highlighting the significant of understanding the local tissue response to hydatid infection to ameliorate diagnostic and treatment strategies (De Biase et al., 2023).

Programmed death-ligand 1 (PD-L1) is a checkpoint molecule of the immune system that can be expressed in the surrounding environment of hydatid cysts, modulating immune responses by inhibiting T-cell activity to prevent excessive tissue damage. Although direct studies on PD-L1 in hydatid lung cysts are limited, its general role in parasitic infections involves the impairment of T-cell responses, which may facilitate parasite survive by evading host immunity. Thus, PD-L1 expression could contribute to the equilibrium between an effective immune response and immune evasion by the parasite, influencing the histological and immunohistological alterations observed in infected lung tissue (Gutic et al., 2023).

Using a mouse model, it was suggested that the parasite load diminished as a result of the effectiveness of a PD-L1 inhibitor. This PD-L1 blockade promoted T-cell activity by elevating CD4+/CD8+ effector T cells and reducing regulatory T (Treg) cells. It also had the potential to restore innate immunity, represented by dendritic cells (DCs) and Kupffer cells/macrophages, and inhibit natural killer (NK) cells. This resulted in the control of *E. multilocularis* infection in mice, implying that the PD-L1 pathway is crucial for regulating both adaptive and innate immune responses to *E. multilocularis* infection, with a considerable impact on tissue inflammation modulation (Jebbawi et al., 2021; Wang et al., 2022). Sun et al. (2024) confirmed that *E. multilocularis* modulates T-cell function via PD-1/PD-L1- and CTLA-4-dependent pathways. Recently, studies have reported that both hydatid cysts and tumors may exploit regulatory T cells (Tregs) and PD-1/PD-L1 pathways to evade immune clearance.

This study was designed to outline the histological changes observed in sheep lung tissue caused by hydatid cysts, assess the localized immune reaction, emphasizing the infiltration of $CD4^+$/$CD8^+$ T-cells and the expression of PD-L1, examine a possible association between hydatid infection and the onset of alveolar adenocarcinoma, and clarify the mechanisms of immune evasion (for instance, T-cell exhaustion and apoptosis mediated by PD-L1) that allow for the persistence of cysts.

Materials and Methods

Sample collection

The College of Education for Pure Sciences Ethic Committee reviewed and approved the current study protocol on April 4, 2024. For the period from September 10, 2024 to April 15, 2025, both infected lungs with hydatid cysts and non-infected lungs were obtained from government slaughterhouses and butchers in local markets of Diyala Governorate. Twenty-four infected lungs and ten non-infected lungs were transferred directly to the Zoology Laboratory in the College of Science at the University of Diyala.

Evaluation of hydatid cyst fertility and protoscolex vitality

The fertility and vitality of the cysts were examined by withdrawing fluid from the cysts. The lungs were sterilized with 70% ethyl alcohol, and then the fluid was withdrawn using a medical sterile needle. It was examined microscopically by placing a drop of the fluid on a glass slide to confirm the presence of protoscoleces. The presence of protoscoleces was confirmed, and their vitality was examined using eosin staining under an Olympus light microscope.

Histology test

Histological sections were routinely obtained after obtaining formalin-fixed paraffin-embedded tissue blocks. Hematoxylin-eosin, Masson trichrome, or periodic acid-Schiff (PAS) stains were used. Histological sections were imaged using a digital camera attached to the microscope.

Immunohistological (IHC) tests

Paraffin-embedded tissue blocks were taken and used for immunohistological examination. Tissue sections, measuring 4–5 µm in thickness, were prepared from all samples, placed onto positively charged slides, and then dried by autoclave. The procedure involved utilizing a ready-to-use mouse monoclonal CD8 antibody (Ref: N1592) and a ready-to-use mouse monoclonal CD4 antibody (Ref: IR649), both sourced from DAKO, Texas, USA. Additionally, the PDL-1 antibody was acquired from Santa Cruz Biotechnology, Texas, USA, and diluted to a concentration of 1:200 (Cat. #sc-8022). The specimens underwent heat-mediated antigen retrieval for 20 minutes by immersion in a high-pH Tris–Ethylenediaminetetraacetic acid (EDTA) solution (Dako, Ref K8000, Glostrup, Denmark). The sections were incubated with primary antibodies overnight at 4 ˚C. A negative control was included in the experimental design.

The cases were assessed based on positive or negative expression. Positive expression was defined as the presence of any immune cells showing positive cytoplasmic staining for CD4, CD8, and PDL-1 antibodies. The percentages of CD4+ and CD8+ T cells, along with PDL-1, were evaluated in five representative high-power fields (HPFs) using a 40x lens (0.344 mm² field of view). The percentage of labeled immune cells relative to total cells was categorized (with small modulate by the pathologist) as: absent (-ve) (1-10% of cells), mild (+ve) (11-30% of cells), moderate (++ve) (31-50% of cells), and marked (+++ve) (>51% of cells) reactions (Dong et al 2002). Two independent pathologists reviewed the results and recorded them as the mean percentage of positive cells (relative to the total cells) per HPF. All microphotographs were captured using a light microscope (Olympus BX51DIC, Japan) attached to a digital camera.

Statistical tests

Statistical tests were performed using SPSS software (version 23) to compare three histological sections for each variable. These sections were made from each lung for both the infected and uninfected study groups. The percentages of lungs showing histological changes were calculated, along with the percentage for each immune expression. A chi-square test was used for comparisons between groups.

Results

Among positive samples for hydatid cysts, about one-third of the samples with hydatid cysts (9 out of 24, 36%) were accompanied by cancerous lesions. The remaining positive samples, constituting about two-thirds of the total (64%), were without adenocarcinoma, as shown in Table 1. This study included 10 non-infected lungs (normal lungs).

In the context of immune marker expression

CD4 expression: 80% of normal lungs were negative for CD4 expression, while 20% showed low expression. In infected lungs, both those without adenocarcinoma (52.6% with low expression) and those with adenocarcinoma (36.8% with low expression and 100% showing moderate expression) demonstrated a tendency towards higher expression compared to normal lungs. There was a significant difference in CD4 expression among normal and infected lungs.

CD8 expression: Low CD8 expression was observed in 47.1% of lungs without adenocarcinoma and in 41.2% of lungs with adenocarcinoma. Moderate expression was found in 50% of lungs without adenocarcinoma and 33.3% of lungs with adenocarcinoma. A significant difference in CD8 expression was noted among the groups.

PD-L1 expression: There was a tendency for PD-L1 expression to be higher in infected lungs than in uninfected lungs. Furthermore, PD-L1 expression tended to be significantly higher in lungs with adenocarcinoma, as detailed in Table 1.

Table 1. Expression of CD4, CD8, and PDL-1 in infected and non-infected lungs

Markers		Groups		CD4			Total
				Negative	Positive	Strongly Positive	
CD4	Group	Normal	Count	8[a]	2[b]	0[b]	10
			% Within-group	80	20	0	100
			% Within CD4	72.7	10.5	0	28.6
		Without adenocarcinoma	Count	1[b]	10[a]	5[a]	16.
			% Within-group	6.3	62.5	31.3	100
			% Within CD4	9.1	52.6	100	45.7
		With adenocarcinoma	Count	2[b]	7[a]	0[b]	9
			% Within-group	22.2	77.8	0	100
			% Within CD4	18.2	36.8	0	25.7
		Total	Count	11	19	5	35
			% Within-group	31.4	54.3	14.3	100
			% Within CD4	100	100	100	100

		Groups		CD8			Total
				Negative	Positive	Strongly Positive	
CD8	Group	Normal	Count	7[a]	2[b]	1[b]	10
			% Within-group	70	20	10	100
			% Within CD8	58.3	11.8	16.7	28.6
		Without adenocarcinoma	Count	5[a]	8[a]	3[b]	16
			% Within-group	31.3	50	18.8	100
			% Within CD8	41.7	47.1	50	45.7
		With adenocarcinoma	Count	0[b]	7[a]	2[b]	9
			% Within-group	0	77.8	22.2	100
			% Within CD8	0	41.2	33.3	25.7
		Total	Count	12	17	6	35
			% Within-group	34.3	48.6	17.1	100
			% Within CD8	100	100	100	100

PDL	Groups			PDL			Total
				Negative	Positive	Strongly Positive	
PDL	Group	Normal	Count	9[a]	1[a]	0[b]	10
			% Within-group	90	10	0	100
			% Within PDL	40.9	12.5	0	28.6
		Without adenocarcinoma	Count	13[a]	3[a]	0[b]	16
			% Within-group	81.3	18.8	0	100
			% Within PDL	59.1	37.5	0	45.7
		With adenocarcinoma	Count	0[b]	4[a]	5[a]	9
			% Within-group	0	44.4	55.6	100
			% Within PDL	0	50	100	25.7
		Total	Count	22	8	5	35
			% Within-group	62.9	22.9	14.3	100
			% Within PDL	100	100	100	100

Note: Different litters vertically mean significant differences at the level of 0.05.

All infected lungs showed numerous lesions, including necrosis, inflammation, fibrosis, and tissue destruction, as show in Table 2, while no lesions were observed in normal lungs.

Histopathological alterations in hydatid infection

The present study showed that in normal sheep lungs stained with hematoxylin-eosin (H&E) stain, the structure of the alveoli, bronchi, and serous membrane appears intact, with normal mucosal thickness and an absence of inflammatory cells. In contrast, sections of infected sheep lungs with hydatid cysts displayed tissue necrosis affecting the alveoli, the cyst wall, and numerous inflammatory cells. The laminated layer of the cyst was surrounded by inflammatory cells, and tissue destruction was evident in the lung, as illustrated in Figure 1A, 1B, and 1C.

Association between hydatid infection and alveolar adenocarcinoma

When using PAS stain on normal and infected sheep lungs (Figures 2, 3, and 4), the observations were as follows: Normal lungs displayed multi-dead space areas (emphysematous bullae) and alveolar macrophages. In infected sheep lungs, alveolar adenocarcinoma of lung tissue was observed (Figure 2B, Figures 3A, 3B, and

Table 2. Histological changes and CD4, CD8 and PDL-1 expression in infected lungs with or without adenocarcinoma

Histological Changes -Adenocarcinoma (n=16) +Adenocarcinoma (n=9)		No. (%)					
		Inflammation		Necrosis		Fibrosis	
		Negative	Positive	Negative	Positive	Negative	Positive
CD4	-Adenocarcinoma	2(12.5)	14(87.5)	11(68.75)	5(31.25)	3(18.75)	13(81.25
	+Adenocarcinoma	0	8(88.89)	2(22.22)	6(66.67)	1(11.11)	7(77.78)
CD8	-Adenocarcinoma	6(37.5)	10(62.5)	10(62.5)	6(37.3)	2(12.5)	14(87.5)
	+Adenocarcinoma	1(12.5)	7(77.78)	2(25)	6(66.67)	1(12.5)	7(77.78)
PDL1	-Adenocarcinoma	3(18.75)	13(81.25)	6(37.5)	10(62.5)	3(18.75)	13(81.25)
	+Adenocarcinoma	1(11.11)	7(77.78)	1(11.11)	7(77.78)	0	8(88.89)

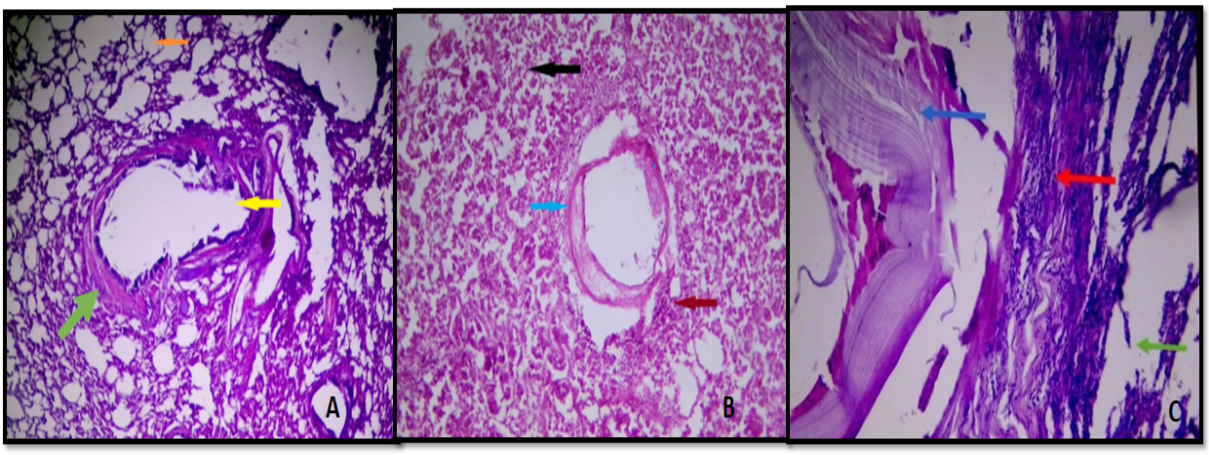

Figure 1. Comparison of histological changes between a normal (uninfected) lung and a lungs infected with hydatid cysts using H & E stain

A) Section of normal sheep lung showing alveoli (orange arrow), bronchi (yellow arrow), and serous membrane (green arrow) (×40), B) Section of infected sheep lung showing necrosis of lung tissue (black arrow), hydatid cyst wall (blue arrow), and inflammatory cells (brown arrow) (×10), C) Section of infected sheep lung showing a thick laminated layer (blue arrow), inflammatory cells around the cyst (brown arrow), and destruction of lung tissue (green arrow) (×40)

3C), along with destruction of the alveolar space (Figure 3B). All these sections (Figures 2, 3, and 4) showed depletion of glycogen in the infected lungs. Reviewing the results presented in the Figures 2, 3, and 4, and the histological sections of infected and non-infected lungs, it was found that more than half of the samples exhibited adenocarcinoma. This finding supports a direct link between infection and adenocarcinoma, suggesting that hydatid cysts may induce this type of cancer.

In Figures 5, and 6, 70% of histological sections of infected lungs stained with Masson's trichrome showed fibrosis and bronchial inflammation. In addition, 55% of the samples showed enlargement of the bronchi surrounded by fibrosis. More than 50% of the samples showed adenocarcinoma, and 90% of the samples showed necrosis, as shown in Figure 6B.

Association between hydatid infection and alveolar adenocarcinoma and immune response

In Figure 7, PD-L1 expression in the lungs of the control group appears to be low in 50% of samples and absent in the rest (Figure 7A). In lungs of sheep infected with hydatid cysts, PD-L1 expression shows low expression in 80% (Figure 7B). Furthermore, there is moderate PD-L1 expression in 90% of lungs from sheep infected with both hydatid cysts and adenocarcinoma (Figure

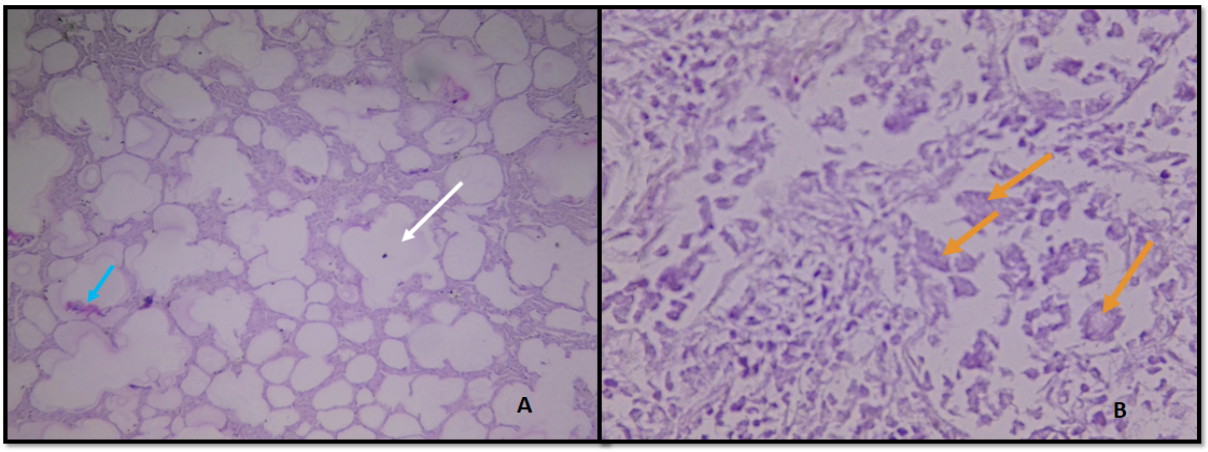

Figure 2. Comparison of histological changes between a normal (uninfected) lung and a lungs infected with hydatid cysts using PAS stain

A) Section of normal sheep lung showing multi-dead space areas (emphysematous bullae) (white arrow) and alveolar macrophages (×10), B) Section of infected sheep lung showing alveolar adenocarcinoma of lung tissue (orange arrow) (×10)

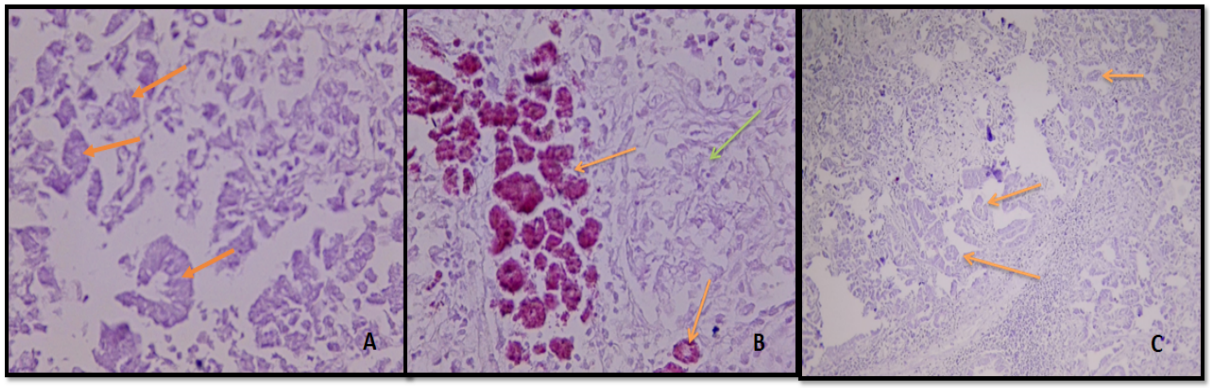

Figure 3. Comparison of histological changes between a normal (uninfected) lung and a lungs infected with hydatid cysts using PAS stain

A) Section of infected sheep lung showing alveolar adenocarcinoma of lung tissue (orange arrow) (×40), B) Section of infected sheep lung showing alveolar adenocarcinoma of lung tissue (orange arrow) and destruction of alveolar spaces (green arrow) (×10), C) Section of infected sheep lung showing alveolar adenocarcinoma of lung tissue (orange arrow) (×4)

7C), which seems to correlate with pleural invasion. Although PDL-1 is expressed in infected lungs with hydatid cysts, in those with both hydatid cysts and cancerous tissue, and in normal lungs, it is more highly expressed in cancerous lungs than in normal ones or those infected only with hydatid cysts.

CD8 expression was observed in the bronchial walls of 40% of infected sheep lungs with hydatid cysts and in the bronchial walls of 70% of lungs infected with both hydatid cysts and adenocarcinoma. In contrast, no CD8 expression was detected in any of the non-infected sheep lungs, as shown in Figures 8A, 8B, and 8C.

In Figure 9, moderate CD4 expression was observed in 75% of sheep lungs infected with hydatid cysts. It was also observed in 60% of lungs infected with both hydatid cysts and adenocarcinoma, while CD4 expression was absent in all non-infected sheep lungs. This indicates an enhanced T cell-mediated immune response in hydatid infection.

Discussion

Results from H&E staining revealed numerous changes, including inflammation, fibrosis, necrosis, and tissue destruction, and this agrees with Abed and Albayati (2019), who revealed that hydatid cyst infection induced infiltration of inflammatory cells and necrosis in the livers of sheep and cows, and with Abo-Aziz et al. (2019), who reported that the lungs of camels showed a fibrous layer, inflammatory reaction, and necrosis. The presence of inflammatory cells in infected tissues confirmed that

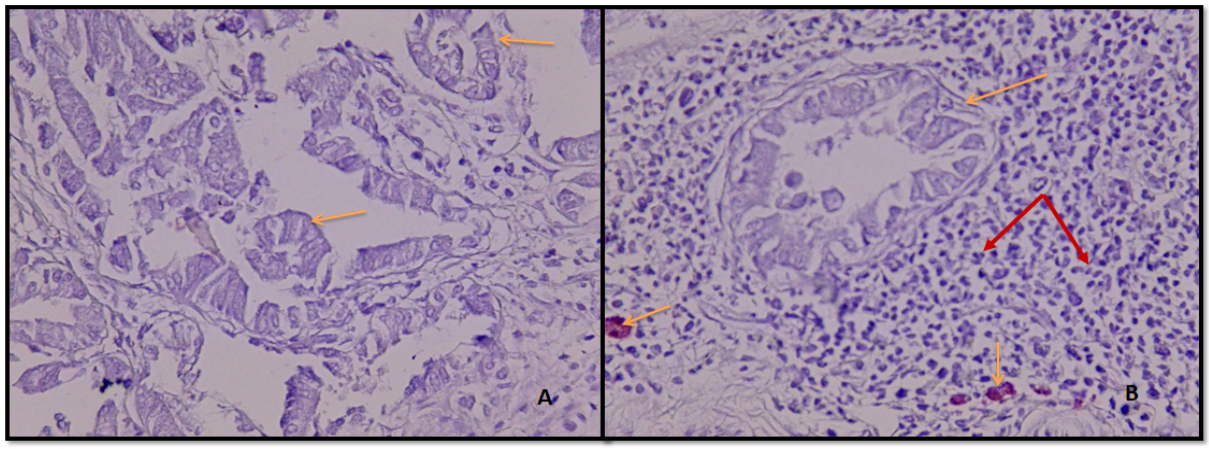

Figure 4. Comparison of histological changes between a normal (uninfected) lung and a lungs infected with hydatid cysts using PAS stain

A) Section of infected sheep lung showing alveolar adenocarcinoma of lung tissue (orange arrow) (×10), B) Section of infected sheep lung showing alveolar adenocarcinoma of lung tissue (orange arrow) and inflammatory cells (brown arrow) (×40)

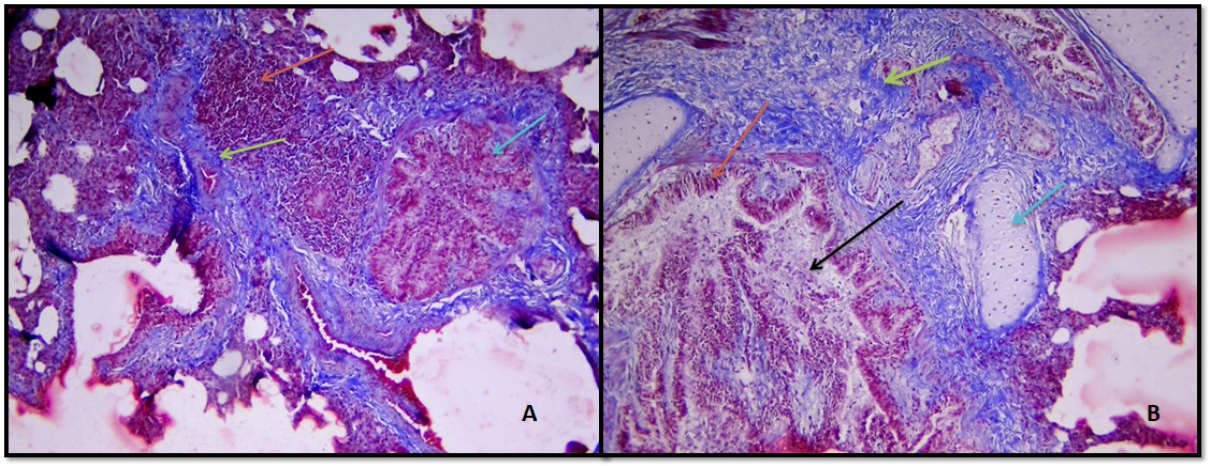

Figure 5. Comparison of histological changes between a normal (uninfected) lung and a lungs infected with hydatid cysts using MASSONS stain

A) Section of infected sheep lung showing increased fibrous tissue (green arrow) and bronchi duct (blue arrow), and inflammation cells (brown arrow) (×10), B) Section of infected sheep lung showing increased fibrous tissue (green arrow) and large bronchi (black arrow), respiratory epithelium (brown arrow), and cartilage (blue arrow) (×10)

infection with hydatid cysts induces inflammation and structural damage. The growth of a cyst depends on the softness of the tissue. Therefore, the cyst grows faster in the lung than in the liver due to the elasticity of lung tissue, putting pressure on the surrounding tissue, and leading to its destruction. Also, the cyst, as a foreign organism, stimulates the infiltration of immune cells, especially inflammatory cells, into the site where it is located. In addition, the presence of cysts surrounded by a fibrous capsule and the destruction of the alveolar tissue structure highlight the host's attempt to contain the parasite as a protective immune response, at the cost of tissue structural integrity (Al-Bermani & Al-Dabhawi, 2022).

In sections stained with PAS and Masson's trichrome stains, adenocarcinoma was observed. Although Turhan et al. (2015) indicated the existence of an immunological link between cancer and immune cysts, which may allow cancer to flourish and spread among organs, some studies have indicated the preventive role of hydatid cysts against cancer (Hosseini et al., 2023).

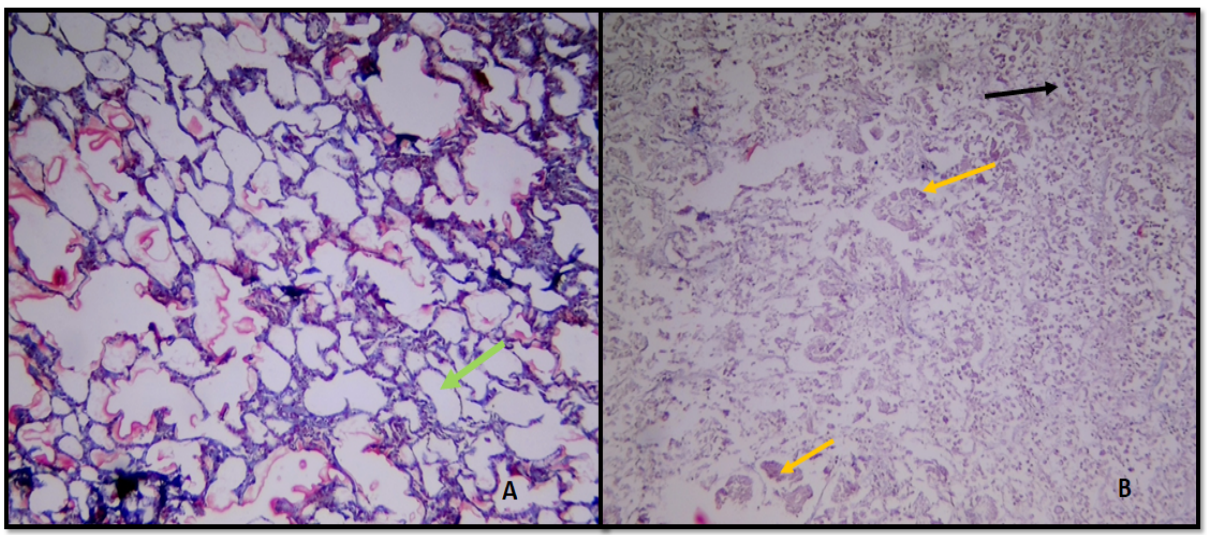

Figure 6. Comparison of histological changes between a normal (uninfected) lung and a lungs infected with hydatid cysts using MASSONS stain

A) Section of normal sheep lung showing alveoli of lung tissue (green arrow) (Masson's trichrome, ×10), B) Section of infected sheep lung showing alveolar adenocarcinoma of lung tissue (orange arrow) and necrosis (black arrow) (Masson's trichrome ×10)

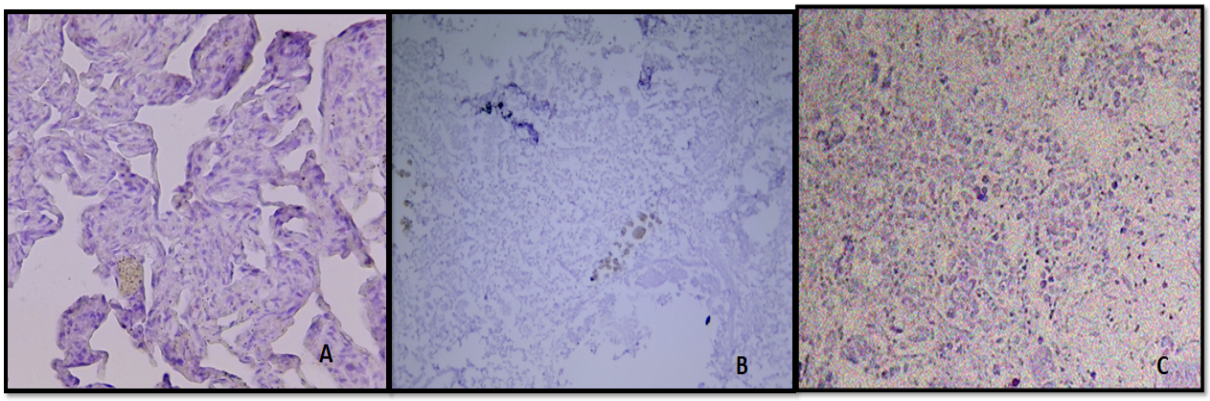

Figure 7. Comparison of histological changes between a normal (uninfected) lung and a lungs infected with hydatid cysts using PDL1 marker

A) PDL1 expression in the lung of the negative control group (×40), B) PDL1 expression in the lung of an infected sheep showing PD-L1 expression (×10), C) PDL1 expression in the lung of a sheep infected with both hydatid cysts and adenocarcinoma (×10)

This relationship is still under investigation, and its mechanism is not yet understood. This highlights the need to comprehend the possible connections between these two conditions better comprehend the possible connections between these two conditions. Research has suggested credible pathophysiological mechanisms linking hydatid cysts to hepatocellular carcinoma (HCC) (Bo et al., 2022), including the possibility that echinococcosis may exert procarcinogenic effects by modulating the host immune response. *E. granulosus* could enhance the growth, migration, and invasive abilities of HCC cells (Yasen et al, 2021). The chronic inflammatory and fibrosis response induced by this parasite in the adjacent liver tissue might contribute to the emergence of HCC (Kovač et al, 2022). In this context, Yagmur et al. (2025) suggested that the shared antigen-receptor framework between *E. granulosus* and cancer could influence immune response signaling, affecting immune system cells and promoting tumor cell progression.

In the current study, there was expression of PD-L1. Munir et al. (2019) referred to the fact that the expression of this protein is associated with the presence of macrophages and may indicate the presence of inflammation. They explained that they were able to link inflammation with PD-L1-specific T cells by showing that inflammatory mediators such as interferon gamma produce measurable numbers of PD-L1-specific T cells, both in vitro and in vivo. These produced cells enhance immune balance by supporting the inflammatory immune response and suppressing regulatory cells. Zhu et al. (2023) demonstrated that PD-L1 expression is related to clinicopath-

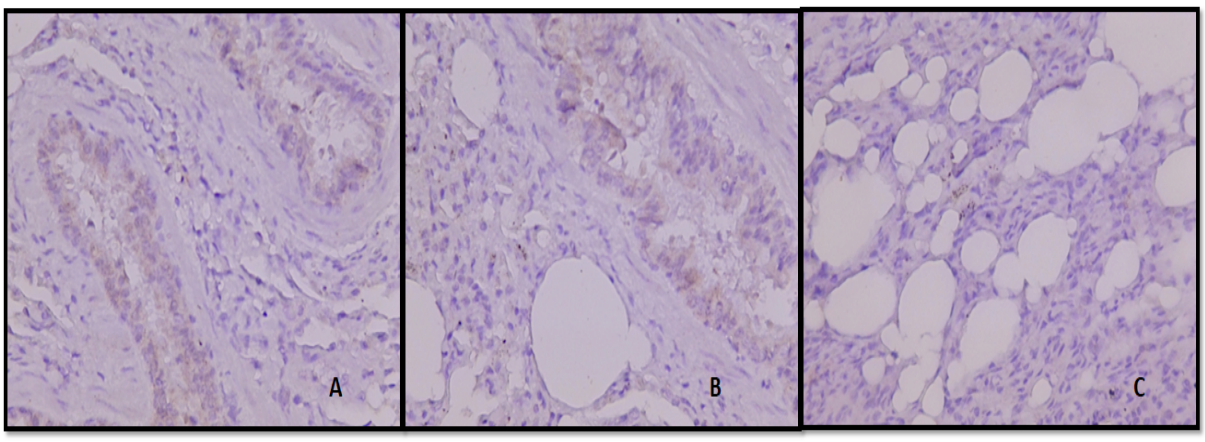

Figure 8. Comparison of histological changes between a normal (uninfected) lung and a lungs infected with hydatid cysts using CD8 marker

A) CD8 expression in the bronchial wall of an infected sheep lung with hydatid cyst, B) CD8 expression in the bronchial wall of the lung in the group infected with both hydatid cysts and adenocarcinoma, C) No CD8 expression in the non-infected lung of a sheep (×40)

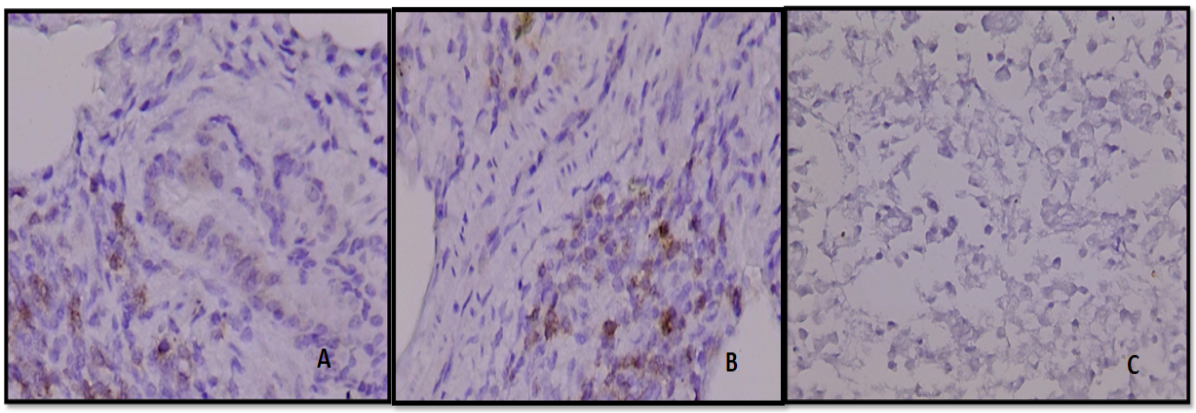

Figure 9. Comparison of histological changes between a normal (uninfected) lung and a lungs infected with hydatid cysts using CD4 marker

A) CD4 expression in infected sheep lung, B) CD4 expression in sheep lung infected with hydatid cysts and adenocarcinoma; C) CD4 expression non-infected sheep lung

ological changes, such as lymphovascular and pleural invasion, which is consistent with current study.

This result highlights T-cell infiltration at infection sites. This is consistent with the point made by Eissa et al. (2024) that, at the level of cytotoxic T cells, CD8+ T cells are stimulated by parasitic infections to harbor more granzymes and perforin, thus increasing their cytotoxic potency. Eventually, parasite infections promoted tumor-selective cytotoxic T-cell invasion and inhibited T regulatory (Treg) cell infiltration into tumor tissue. The remarkable rise in the CD8+ T-cell/Treg ratio is a vital predictive factor in parasitic infections.

Additionally, Zhang et al. (2017) indicate the role of numerous T cell subtypes (such as CD4, CD8, and CD8) in both controlling parasite infection and contributing to liver damage, particularly in high-intensity of infections. Their study also focuses on the relationship between T cell drainage markers. This model presents a valuable tool for studying immunomodulation as a therapeutic approach for alveolar echinococcosis.

$CD8^+$ cytotoxic T lymphocytes (CTLs) are critical in eliminating and controlling intracellular pathogens and malignant cells. In this study, $CD8^+$ T cells were particularly concentrated around hydatid cysts and within the bronchial walls of infected lungs with cysts, indicating an active immune response against *E. granulosus* (Figure 8A and B). Their presence may correlates with granzyme B expression, as mentioned by Hamad et al. (2024), suggesting cytotoxic activity to control cyst survival.

However, the synchronous upregulation of PD-L1 (Figures 7B and 7C) suggests a conversely regulatory mechanism. PD-L1, expressed by cyst-associated and cancerous tissues, engages PD-1 on CD8 T cells, leading to T cell exhaustion and reduced effector function (Eissa et al., 2024). Also, apoptosis mediated by Fas/FasL activation-induced cell death (AICD) depletes CTLs (Bo et al., 2020). This immunosuppressive microenvironment may explain why hydatid cysts survive despite robust primary $CD8^+$ recruitment and could similarly facilitate immune escape of cancerous growth in adenocarcinoma.

In the study by Wang et al. (2018), which is consistent with the present study, in infected mouse models, the Treg immune response was decreased following the use of anti-PD-L1. Tregs (CD4+ Foxp3+) generate a highly immunosuppressive environment that promotes tumor growth by maintaining PD-1 expression on their surface. In the presence of CD3 and TGF-β, the PD-1 receptor on Treg cells increases the transformation of naive CD4+ T cells into Treg cells, thereby reducing immune effector responses. This process increases Treg expression and immunosuppressive function of CD4+ T cells. Consequently, the presence of PD-1 expression not only suppresses effector T cell function but also promotes the conversion of the immunosuppressive Treg cell population.

There is an increasing amount of research focusing on the role of CD4+ T cell responses against tumors. CD4+ T cells play a responsible role in cytokine production and T-cell regulation. A previous study observed that local recruitment of CD4+ T cells is associated with longer overall survival (OS) and disease-specific survival (Gettinger et al., 2015). Similarly, in the study by Niemeijer

et al. (2020), the presence of local CD4+ T cells was also linked to better OS in cancer patients.

The expression of PD-L1, CD3, CD4, and CD8 is correlated with immune response in tumor patients. However, the expression of CD8+ T cells and PD-1 did not show a significant correlation with response in the study conducted by Sahba et al. (2017) They concluded that there is a positive correlation between PD-L1 expression and response to therapy. Additionally, the presence of CD4 expression identified positive macrophages for PD-L1, and each of stromal PD-L1, CD3, CD4, and CD8 immune cell (IC) expression was considered a potential predictive marker for treatment response.

In the context of hydatid cyst infection, this pathway likely contributes to immune evasion by the parasite, as PD-L1 engagement suppresses T cell activity, reducing effective immune clearance. CD8+ T cells, which are more sensitive to PD-1/PD-L1 inhibition due to their limited IL-2 production, can undergo apoptosis through pathways, such as Fas/FasL-mediated AICD, where Fas ligand expressed on T cells triggers apoptosis in neighboring T cells. This mechanism depletes cytotoxic CD8+ T cells, further dampening the immune attack on the cyst.

Moreover, hydatid cyst fluid itself can induce lymphocyte apoptosis, helping the parasite escape immune destruction. The combined effect of PD-1/PD-L1 signaling and Fas/FasL interactions leads to T cell apoptosis and immune suppression, facilitating cyst survival and persistence in lung tissue. Thus, CD4+ and CD8+ T cells are regulated by PD-L1 to limit excessive inflammation, but this also inadvertently allows hydatid cysts to evade host immunity by inducing T cell apoptosis.

Conclusion

This study demonstrates a significant link between pulmonary hydatid cyst infection and the growth of alveolar adenocarcinoma in ovine lung tissue. Extensive tissue damage was confirmed by histopathological tests, including inflammation, necrosis, and fibrosis, while immunohistological results revealed vigorous CD4+ and CD8+ T-cell infiltration, indicative of an effective immune response against parasitic infection. Notably, PD-L1 expression was significantly elevated in adenocarcinoma-associated samples, suggesting a possible mechanism of immune evasion that parallels processes of tumorigenesis.

The concurrent presence of hydatid cyst infection and adenocarcinoma raises the hypothesis that chronic parasitic infection may provoke a pro-inflammatory and immunosuppressive microenvironment that contributes to malignant transformation. The PD-L1 upregulation in infected and cancerous tissues supports the hypothesis that *E. granulosus* uses immune checkpoint pathways to evade host defenses, similar to mechanisms observed in cancer immune evasion.

These results contribute to the growing body of evidence associating parasitic infections with oncogenesis and confirm the need for further studies into the molecular mechanisms of this relationship. Future research should investigate targeted immunotherapies, such as PD-L1 inhibitors, as possible dual-treatment strategies for hydatid disease and associated malignancy.

Ethical Considerations

Compliance with ethical guidelines

Ethical approval for this study was obtained from the Ethical Review Board of the College of Science/University of Diyala, Diyala, Iraq (Code: CEPEC/020).

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Authors' contributions

Sample collection and investigation: Asraa Dawod Farhan; Conceptualization, study design, and writing the original draft: Nagham Y. Albayati; Formal analysis and fnal approval: All authors.

Conflict of interest

The authors declared no conflict of interests.

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