

Original Paper

Histopathological and Immunobiochemical Alterations Associated with Natural *Fasciola hepatica* Infection in Sheep: Emphasis on IL-10–Mediated Immune Modulation

Shahad Mohammed Mahmood

Department of Pathological Analysis, College of Applied Sciences, University of Samarra, Iraq

Correspondence email | Shahadalsalam6@gmail.com

Received: 27/06/2026

Accepted: 30/06/2026

Available online: 30/09/2026

DOI: <https://doi.org/10.63964/atmj.2026.2.3.2>

ABSTRACT

Background: Fasciolosis is a significant helminthic disease of sheep, affecting the liver and bile ducts, and causing economic losses. *Fasciola hepatica* has been demonstrated to induce a regulatory immune response in the host, with an important role for the cytokine interleukin-10 (IL-10). The primary objective of this study was to characterise the histopathological, haematological, biochemical and immunological changes in natural infected sheep infected with *F. hepatica*, focusing on the level of IL-10 in the serum and its correlation with hepatobiliary damage. Thirty local breed sheep (1-3 years old) were examined in Samarra Municipal Abattoir, Salah Al-din Province, Iraq, from November 2025 to February 2026 and were divided into naturally infected group (n=15) and healthy control group (n=15). Diagnosis of infection was made by faecal sedimentation and Ure 1988's recovery of adult flukes from the bile ducts. Haemoglobin (Hb), liver enzymes (AST, ALT, ALP, GGT) and serum IL-10 (ELISA) were analyzed and liver tissue was assessed by H&E and Masson's trichrome staining. Student's t-test and Pearson's correlation were used to analyse data. Results: The level of Hb was significantly reduced in the infected group (8.74 ± 0.63 g/dL vs 12.49 ± 0.39 g/dL) and all liver enzymes were significantly elevated in the infected sheep ($P < 0.001$), with the greatest increases observed for the cholangiocellular markers GGT (+143%) and ALP (+102%). Serum IL-10 was markedly elevated in infected animals (46.20 ± 5.37 vs. 19.13 ± 1.85 pg/mL; $P < 0.001$) and correlated very strongly with GGT ($r = 0.994$), ALP and ALT ($r = 0.983$), and AST ($r = 0.964$), and inversely with Hb ($r = -0.616$). Histopathology confirmed eosinophilic infiltrates around the portal areas, hyperplasia of the bile ducts, cholangitis and significant fibrosis. In conclusion, natural infection of sheep with *F. hepatica* causes predominantly cholangiocellular liver damage in sheep, which is accompanied by an IL-10-driven immunomodulatory response and anaemia. The hepatobiliary damage in both IL-10 and disease severity is well correlated, indicating that both processes are tightly coupled with infection intensity, and thus, the usefulness of IL-10 as an auxiliary marker of disease severity in ovine fasciolosis. It is important to note that due to the small sample size and correlational design, these results are preliminary.

KEYWORDS: *Fasciola hepatica*; sheep; IL-10; immune modulation; histopathology; liver enzymes; Iraq.

1. INTRODUCTION

Fasciolosis, caused by the liver flukes *Fasciola hepatica* and *Fasciola gigantica*, is one of the most important helminthic diseases of ruminants worldwide, responsible for substantial economic losses through reduced productivity, liver condemnation, and mortality.¹ It is classified by the World Health Organization as a neglected tropical disease and is recognised as a re-emerging food-borne zoonosis, with an estimated 2.4 million people infected and many millions more at risk.² A recent global meta-analysis estimated pooled prevalences of approximately 17% in cattle and 13% in sheep, underscoring the scale of the problem in small ruminants.³ In sheep, the disease is of particular significance owing to the marked susceptibility of this host and the severity of the hepatic damage that ensues.⁴ Humid conditions in Iraq and its neighbours are conducive to the intermediate snail host, which leads to a high prevalence of fasciolosis in grazing livestock in these areas. Several studies have reported on the disease in sheep, describing the occurrence of established infection and typical hepatic histopathology of

slaughtered sheep⁵, and the development of anthelmintic resistance in sheep in Iraq.⁶ This has emphasised the durability of the disease as well as the fact that the control measures have depended mainly on chemotherapy.

Adult flukes cause mechanical and inflammatory damage to the bile duct and the pathogenesis of ovine fasciolosis is focused on the bile duct. There is a relationship between the measurable biochemical changes (elevations in cholangiocellular enzymes gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP), as well as the hepatocellular enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and the lesions resulting from this; however, blood loss and inflammation cause haematological changes that can also be informative in the evaluation of disease.^{7,8}

In addition to these structural and biochemical modifications, *Fasciola* species are also known to actively regulate the host immune response. For the parasite to persist in the host, it induces a polarization against its host-protective Th1 response towards a Th2 and regulatory phenotype, in which anti-inflammatory cytokines, in particular, interleukin-10 (IL-10), play a central role.⁹ Investigations in sheep have also been undertaken to relate variation in fluke burden with expression of immunological markers, including those of regulatory cytokines including IL-10 and TGF- β 1,¹⁰ and more recently local histological studies have shown the presence of globule leukocytes and mast cells in the bile ducts of chronically infected sheep,¹¹ suggesting that effector cells associated with the Th2 pathway are also involved in the biliary environment.

However, integrated studies encompassing the four aspects of a natural ovine fasciolosis (parasitological, haematological, biochemical, histopathological, and immunological) coupled with the direct examination of the role of IL-10 in the severity of the hepatobiliary injury are still limited, especially in the Iraqi context. The present study aimed therefore to look at the histopathological and immunobiochemical changes that occurred during natural *F. hepatica* infection in sheep, particularly regarding the level of modulation of the immune system as indicated by the concentration of IL-10 in the blood, and to evaluate the correlation of this parameter with other parameters associated with hepatobiliary damage and anaemia.

2. MATERIALS AND METHODS

2.1. Study Design and Animals

The total number of sheep in this case-control study was thirty ($n = 30$), broken down into two groups comprising 15 naturally *F. hepatica*-infected sheep and 15 sheep that appeared to be healthy. To minimise confounding factors, the age and sex distribution of the sampled sheep was similar in both groups, ranging from about 1–3 years of age, and weighing from 28 to 45 kg. Patients had to be confirmed as having patent infection by faecal sedimentation and recovery of adult flukes from the bile ducts at necropsy to be included in the infected group. Animals were excluded from the study if they had concurrent hepatic disease (such as hydatid cysts, abscesses) other than fasciolosis, signs of other systemic disease or had been treated with an anthelmintic within the previous 48 hours. There was a requirement for the control animals to be negative upon faecal examination and liver inspection. The study protocol has been approved by the Animal Ethics Committee (approval number USC-Bio-2025-011), College of Applied Sciences at University of Samarra.

2.2. Study Area and Period.

Samples were taken from Samarra Municipal Abattoir in Salah Al-Din Province, Iraq from 2025/11 up to 2026/2. This time of year coincides with the peak period of the season at which transmission and adult fluke burdens are the highest for the region, and is when ovine fasciolosis is at its highest level in the region.

2.3. Ante-mortem and post-mortem inspection.

Routine Veterinary exam of all animals which included ante-mortem and post-mortem examination. Macroscopic examination of livers was performed at necropsy for the presence of gross lesions typical of fasciolosis, such as thickening of the bile duct, fibrosis and presence of adult fluke. Adult *F. hepatica* were recovered from the hepatic bile ducts, confirming the infection, flukes recovered were washed in physiological saline and identified by their characteristic leaf-shaped body, anterior cephalic cone and branched intestinal ceca.

2.4. Coprological Examination (Sedimentation)

Faecal samples were obtained directly from the rectum of each animal and the eggs of *F. hepatica* were recovered by the standard sedimentation technique. Operculated large, golden-brown eggs, observed under light microscopy ($\times 40$ objective) were regarded as diagnostic of patent infection.

2.5. Blood Sampling and Haematology

Blood samples were drawn from the jugular vena from each animal. Part of it was transferred to EDTA tubes for a complete blood count (CBC) and the haemoglobin (Hb) concentration was measured on a Mindray BC-2800Vet Hematology Analyzer

(China). The second sample was collected in plain tubes, allowed to clot, then centrifuged at 3000 rpm for 10 minutes to get serum that was stored at -20°C until biochemical and immunological analysis.

2.6. Serum Liver Enzyme Assays

The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) activities were determined spectrophotometrically, following manufacturer's instructions (Biolabo, France). GGT and ALP were particularly evaluated, as indicative of bile duct (cholangiocellular) damage, typical of the fasciolosis.

2.7. Serum Interleukin-10 (IL-10) was determined by the Elisa method. Serum Interleukin-10 (IL-10) was measured by Elisa method.

IL-10 levels were determined in serum using a commercially available ELISA kit which was specific for sheep (Sheep IL-10 ELISA Kit, MyBioSource, USA) according to the manufacturer's instructions. Each sample was analysed twice. Absorbance measurements were taken at 450 nm by using a microplate reader and the IL-10 concentrations were interpolated from a standard curve and reported in pg/mL. All measured values were within the linear detection range of the assay and the intra- and inter-assay coefficients of variation were below the manufacturer's specifications ($< 10\%$ and $< 12\%$, respectively).

2.8. These are done by examining the tissue under a microscope and assigning an overall score.

Representative samples of liver tissue were taken from the affected lobes, fixed in 10% neutral buffered formalin, processed in graded ethanol, cleared in xylene and paraffin embedded. The evaluation of inflammatory and degenerative changes in the tissues was done on sections of 4-5 μm thickness stained by haematoxylin and eosin (H&E) while hepatic fibrosis and collagen deposition were evaluated on sections of 4-5 μm thickness stained by Masson's trichrome. The stained areas were observed using a light microscope at magnifications of $\times 100$, $\times 400$ and $\times 1000$ and photographs were taken to document their observations.

The severity of the lesions was assessed by an examiner who was blinded to the group allocation, on four parameters (portal and periportal inflammatory infiltration, bile duct hyperplasia, eosinophil density, hepatic fibrosis in trichrome stained sections) using an ordinal scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). The parameter scores were summarised for each parameter and compared descriptively to the control group where these lesions always did not occur.

2.9. Statistical Analysis

Data are presented as mean $\pm$ SD. The normality of distribution for each parameter was tested by Shapiro–Wilk test and the results showed that all the parameters were normally distributed in both the groups ($P > 0.05$); hence, parametric tests were used. The independent-samples Student's t-test was used to compare the difference between the infected and control group and the size of the difference was reported as Cohen's d effect size along with 95% confidence interval (CI) of the difference between the two means. Within the infected group, relationships between serum IL-10 concentration and haematological and biochemical parameters were assessed by Pearson's correlation coefficient (r) and an additional non-parametric analysis of robustness, Spearman's rank correlation (ρ), was performed. p values less than 0.05 were deemed to be statistically significant. The analyses were done in regular statistical software (e.g., IBM SPSS Statistics v. 26).

3. RESULTS

3.1. The results of parasitological and Macroscopic findings are also reported.

All of the infected group were diagnosed as patent *Fasciola hepatica*. The characteristic large, golden-brown operculated eggs of *F. hepatica* were seen by light microscopy in coprological sedimentation.



Figure 1. *Fasciola hepatica* egg detected in a sheep faecal sample using the sedimentation technique, showing a large, oval, operculated egg; $\times 40$.

3.2. Haematological Findings

The mean concentration of haemoglobin was significantly lower in the infected group than the control group (8.74 ± 0.63 g/dL vs 12.49 ± 0.39 g/dL, $P < 0.001$), which corresponds to about a 30% decrease. This anaemic picture is typical of the chronic blood loss and inflammatory anaemia which occurs in biliary fasciolosis.

3.3. Serum Liver Enzyme Activities

All four liver enzymes were significantly elevated in the infected group relative to controls ($P < 0.001$ for each; Table 1). The most pronounced increases were observed in the cholangiocellular markers: GGT rose by approximately 143% (94.27 ± 8.07 vs. 38.73 ± 2.49 U/L) and ALP by approximately 102% (294.93 ± 29.05 vs. 145.87 ± 6.25 U/L). The hepatocellular enzymes also increased markedly, with ALT and AST rising by approximately 88% and 84%, respectively. The disproportionate elevation of GGT and ALP relative to the transaminases indicates a predominantly biliary (cholangiocellular) pattern of injury, in agreement with the histopathological findings.

3.4. Serum Interleukin-10 (IL-10)

Serum IL-10 concentration was significantly higher in the infected group than in controls (46.20 ± 5.37 vs. 19.13 ± 1.85 pg/mL; $P < 0.001$), corresponding to an approximate 142% increase. This elevation is consistent with the upregulation of anti-inflammatory, regulatory signalling commonly reported in chronic helminth infection; however, as only IL-10 was measured, it should be regarded as an indicator of a regulatory response rather than direct evidence of a full Th2/regulatory polarisation.

Table 1. Haematological and biochemical parameters in control and *F. hepatica*-infected sheep, with effect sizes.

Parameter	Control (n=15)	Infected (n=15)	P-value	Cohen's d	95% CI of diff.
Hb (g/dL)	12.49 ± 0.39	8.74 ± 0.63	< 0.001	-7.18	-4.14 to -3.35
AST (U/L)	72.27 ± 2.81	132.60 ± 9.50	< 0.001	8.61	54.92 to 65.75
ALT (U/L)	31.73 ± 2.37	59.60 ± 5.19	< 0.001	6.90	24.79 to 30.95
ALP (U/L)	145.87 ± 6.25	294.93 ± 29.05	< 0.001	7.09	132.74 to 165.39
GGT (U/L)	38.73 ± 2.49	94.27 ± 8.07	< 0.001	9.30	50.93 to 60.14
IL-10 (pg/mL)	19.13 ± 1.85	46.20 ± 5.37	< 0.001	6.74	23.97 to 30.16

Values are mean $\pm$ SD; group differences assessed by independent-samples Student's *t*-test. All parameters were normally distributed (Shapiro-Wilk $P > 0.05$). Cohen's *d* values indicate very large effect sizes.

3.5. Correlation Analysis

Within the infected group, serum IL-10 concentration showed strong and statistically significant positive correlations with all hepatobiliary enzymes, being highest with GGT ($r = 0.994$, 95% CI 0.981–0.998), followed by ALP and ALT ($r = 0.983$ each).

and AST ($r = 0.964$), all with $P < 0.001$. Conversely, IL-10 was moderately and inversely correlated with haemoglobin ($r = -0.616$, 95% CI -0.857 to -0.151 ; $P < 0.05$). These relationships were confirmed by non-parametric Spearman's rank correlation ($\rho = 0.97-0.99$ for the enzymes; $\rho = -0.57$ for Hb), indicating that the associations were not driven by outliers (Table 2). The raw paired values underlying the strongest association (IL-10 versus GGT) are presented in Table 3 for full transparency. These associations indicate that the magnitude of the IL-10-mediated immune response parallels the severity of hepatobiliary injury and anaemia, suggesting that both the tissue damage and the immunomodulatory response are driven concurrently by the intensity of infection rather than by a direct causal link.

Table 2. Correlation between serum IL-10 and other parameters in the infected group ($n = 15$).

vs. IL-10	Pearson r	95% CI	Spearman ρ	Interpretation
GGT	+0.994	0.981 to 0.998	+0.993	Very strong (+)
ALP	+0.983	0.948 to 0.994	+0.989	Very strong (+)
ALT	+0.983	0.949 to 0.995	+0.993	Very strong (+)
AST	+0.964	0.893 to 0.988	+0.971	Very strong (+)
Hb	-0.616	-0.857 to -0.151	-0.568	Moderate (-)

All enzyme correlations $P < 0.001$; Hb correlation $P < 0.05$. Spearman's ρ provided as a non-parametric robustness check.

Table 3. Paired serum IL-10 and GGT values for individual infected sheep (raw data underlying the strongest correlation).

Animal	IL-10 (pg/mL)	GGT (U/L)
F1	39	84
F2	48	96
F3	51	101
F4	41	88
F5	46	94
F6	37	79
F7	54	105
F8	44	91
F9	49	98
F10	42	87
F11	45	93
F12	55	108
F13	43	90
F14	47	97
F15	52	103

Pearson $r = 0.994$; Spearman $\rho = 0.993$ ($n = 15$).

3.6. Histopathological Findings

The liver sections from the infected and control animals were stained with haematoxylin and eosin and examined histopathologically showing a marked difference between the two groups. Hepatic architecture was normal in all control livers,

and no abnormalities were observed in portal triads, hepatic cords, central veins, bile ducts, or Kupffer cells (Figure 3A, C). In contrast, infected livers at low magnification exhibited prominent lesions such as periportal fibrosis, bile duct hyperplasia, and focal necrosis (Figure 3B), while at high magnification, the bile duct was dilated, periportal immune cell infiltration was dense, there was fibrosis, and scattered apoptotic bodies were observed (Figure 3D). The mixed inflammatory infiltrate was predominantly made up of eosinophils, lymphocytes, plasma cells, and macrophages surrounding dilated and hyperplastic bile ducts, with signs of periductal fibrosis and cholangitis. Liver fibrosis was confirmed on Masson's trichrome staining, which showed the presence of wide fibrous septa (blue staining) connecting portal areas and central vein, along with collagen deposition around the portal and pericellular structures, and the development of nodular architecture (Figure 4). Semi-quantitative scores of the main lesions summarized in Table 4, with all the parameters being moderate to severe in the infected group and always absent in the controls.

Table 4. Semi-quantitative histopathological scoring of liver lesions in the infected group (0 = absent, 3 = severe).

Lesion	Infected (mean score)	Control
Portal/periportal inflammation	2.7 (moderate–severe)	0
Bile duct hyperplasia	2.5 (moderate–severe)	0
Eosinophil density	2.8 (severe)	0
Hepatic fibrosis (trichrome)	2.6 (moderate–severe)	0

Scores assigned by a blinded examiner using a 0–3 ordinal scale; values are group means across infected animals. Lesions were absent in all control livers.

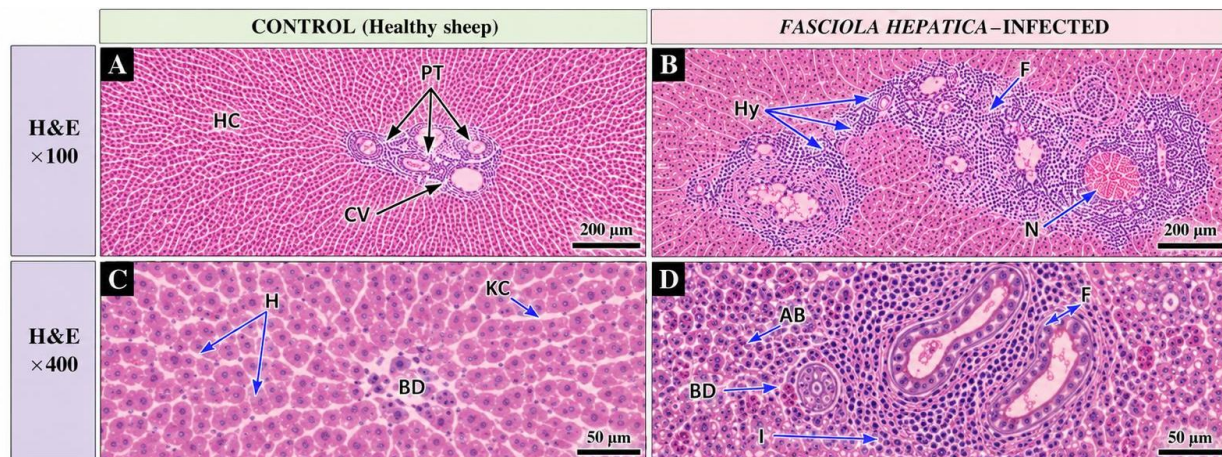


Figure 3. Comparative histopathological alterations in liver sections of control and *Fasciola hepatica*-infected sheep stained with hematoxylin and eosin. (A) Normal liver ($\times 100$) with portal triad (PT), hepatic cords (HC) and central vein (CV). (B) The liver is infected with *Fasciola hepatica*, marked histopathological changes include periportal fibrosis (F), bile duct hyperplasia (Hy) and focal necrosis (N) at $\times 100$. Normal hepatocytes (H), bile duct (BD) and Kupffer cells (KC) in liver section ($\times 400$) (C). Infected liver section at $\times 400$ with dilated bile duct (BD), dense immune cell infiltration (I), fibrosis (F) and apoptotic bodies (AB). Scale bars: 200 μm in A and B; 50 μm in C and D. H&E stain.

4. DISCUSSION

In this study, significant haematological, biochemical, immunological and histopathological changes were recorded in the naturally infected sheep and, significantly, the levels of serum interleukin-10 (IL-10) were strongly correlated with the degree of hepatobiliary injury. Together, these results support the notion that the disease state of ovine fasciolosis is not simply a mechanical injury to the liver, but in fact has the ability to actively remodel the host immune response.

4.1. Haematological Alterations

The marked decrease in haemoglobin in infected animals is similar to that seen in the well recognized anaemia of chronic fasciolosis. This anaemia is multifactorial, due to chronic blood loss into the bile duct by haematophagous activity of the adult fluke, plus the anaemia of chronic inflammation. Haemoglobin was correlated with IL-10 to show the inverse correlation ($r = -0.616$), which indicates that the most affected animals, also the ones with the highest immunomodulatory response, were the ones with the highest erythrocyte depletion, thus correlating immune activation with disease severity.

4.2. Hepatobiliary Enzyme Profile

Liver enzymes are elevated, and there is disproportionately high levels of GGT and ALP compared to the transaminases, suggesting a mostly cholangiocellular (biliary) pattern of liver injury. This is biologically plausible, because in the adult stage *F. hepatica* spends its time inside the bile duct system where its feeding and migration leads to damage of the epithelium, hyperplasia of the bile ducts, and periductal fibrosis. Hepatocellular degeneration of the parenchyma around the area was also evident by the elevated levels of AST and ALT, and confirmed histologically. This enzymatic pattern agrees with previous work from the region which reported on hepatic dysfunction and structural liver damage in slaughtered sheep with fasciolosis,⁵ and experimental and field studies which correlated increased GGT with increased activity of other serum enzymes associated with biliary fluke.^{7,13,14}

4.3. Immune Modulation, IL-10

The major conclusion of this study was the marked increase in serum IL-10 levels in infected sheep and its highly positive correlation with hepatobiliary enzymes. The concurrent increase in IL-10 with biliary injury markers demonstrates that the response of the host to *Fasciola* infection is a regulatory response as well as a tissue injury response, and is related to the strength of the infection.

This is in keeping with experimental immunological studies in sheep, in which the levels of IL-10 and of TGF- β 1 were correlated with changes in the intensity of the infection.¹⁰ In this sheep model, these cytokines would be considered indicators of immune regulation induced by the parasite. The histological evidence of globule leukocytes in the bile ducts of sheep suffering from chronic fasciolosis, along with mast cells, which are also part of the same immunological axis, is a locally complementary cellular basis for the Th2-type, IL-10-associated response reported here.

The extremely high correlation coefficients ($r = 0.96$ to 0.99) should be treated with some care. These relationships do not imply a direct causal relationship between IL-10 and enzyme leakage, but rather that in both cases the animals are more likely to have a higher degree of infection intensity, which causes the animals to have greater damage to the bile (enzymes) and also a greater regulatory cytokine response (IL-10). The observed pattern is the most parsimonious, and is a concurrent escalation, not a cause-and-effect.

4.4. Histopathological Correlates

The biochemical and immunological observations are correlated with the histopathological findings. The cholangiocellular enzyme pattern is directly explained by the dense periportal infiltrate, which is composed mainly of eosinophils, and by the presence of bile duct hyperplasia, cholangitis and periductal fibrosis, which are characteristic features of fasciolosis. The high collagen deposition detected by the Masson's trichrome staining is in line with the presence of a chronic inflammatory and profibrotic milieu that is the morphological endpoint of the action of IL-10 and related regulatory cytokines. The immune milieu was skewed towards the Th2 type with an increase in IL-10, which was confirmed by immune analysis of transcripts and early infection in the sheep.^{17,20} The high magnification presence of eosinophils and activated macrophages confirms the Th2 skewed immune milieu.

4.5. The region and epidemiological context is provided. The region and epidemiological context is provided.

Therefore, the finding of patent infection of *F. hepatica* in sheep slaughtered in Salah Al-Din Province highlights the ongoing veterinary and economic significance of fasciolosis in Iraq. The clinical relevance of biochemical and immunological markers,^{16,19} and the general regional situation of the presence of fasciolosis in neighbouring countries with similar humid environmental conditions,⁴ suggest the importance of integrated diagnostic and immunological approaches like the one used here.

4.6. Limitations

There are some limitations to be noted in this study. Sample size was reasonably appropriate to have identified very significant differences but was relatively small and only from one abattoir at one peak of the season and this could restrict generalisability. Cytokine expression in the tissue would give a much better picture of the Th1/Th2/regulatory balance and a wider cytokine panel, such as IFN- γ , TGF- β , and IL-4, could be measured in the serum. Lastly, it was not possible to quantify the intensity of the infection (number of flukes) per animal, and thus test the infection-intensity hypothesis proposed above directly. These are all good suggestions for further studies.

5. CONCLUSION

In this study, it was shown that natural infection with *Fasciola hepatica* in sheep is a condition with considerable anaemia, with a distinct pattern of elevation of hepatic enzymes, which is predominantly cholangiocellular, marked IL-10 upregulation of regulatory cytokines and characteristic histopathological changes leading to hepatic fibrosis. High correlations between serum IL-10 and hepatobiliary enzymes, and the fact that its level is reduced in the presence of haemoglobin, suggests that the immunomodulatory response and the severity of tissue damage are closely linked, most likely because of their simultaneous detrimental effects as a result of the infection's intensity, but not necessarily causally. The results suggest the use of serum IL-10 as a potential auxiliary diagnostic tool for the clinical diagnosis of ovine fasciolosis and warrant the combined use of parasitological, biochemical, immunological and histopathological approaches to study the host–parasite relationship. The findings are tentative due to the small, single-centre sample, and the correlational design. The immunoregulatory aspects of chronic *F. hepatica* infections should be further explored by future studies that include larger multi-centre cohorts, quantifiable fluke burdens, a more extensive cytokine panel and tissue expression of cytokines.

Declarations

Ethical approval: The study protocol was approved by the Animal Ethics Committee, University of Samarra, College of Applied Sciences (approval number USC-Bio-2025-011).

Conflict of interest statement: There are no conflicts of interest to declare.

REFERENCES

1. Khan MK, Sajid MS, Riaz H, et al. A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. *Microbial Pathogenesis*. 2017;109:253–262.
2. Mas-Coma S, Bargues MD, Valero MA. Fascioliasis and other plant-borne trematode zoonoses. *International Journal for Parasitology*. 2005;35(11–12):1255–1278.
3. Zhang X, Cui J, et al. Global prevalence of liver disease in human and domestic animals caused by *Fasciola*: a systematic review and meta-analysis. *Journal of Global Health*. 2024;14:04223.
4. Ashrafi K. The status of human and animal fascioliasis in Iran: a narrative review article. *Iranian Journal of Parasitology*. 2015;10(3):306–328.
5. Ashoor SJ, Wakid MH. Prevalence and hepatic histopathological findings of fascioliasis in sheep slaughtered in Jeddah, Saudi Arabia. *Scientific Reports*. 2023;13:6609. doi:10.1038/s41598-023-33927-0
6. Marif HF, Rashid ZM, Mama HO, Dyary HO. Resistance of *Fasciola hepatica* to triclabendazole, closantel, and rafoxanide in a sheep farm in Sharazor district, Kurdistan-Iraq. *Basrah Journal of Veterinary Research*. 2019;18(2):16–26.

7. Hodžić A, Zuko A, Avdić R, Alić A, Omeragić J, Jažić A. Influence of *Fasciola hepatica* on serum biochemical parameters and vascular and biliary system of sheep liver. *Iranian Journal of Parasitology*. 2013;8(1):92–98.
8. Matanović K, Severin K, Martinković F, Šimpraga M, Janicki Z, Barišić J. Haematological and biochemical changes in organically farmed sheep naturally infected with *Fasciola hepatica*. *Parasitology Research*. 2007;101(6):1657–1661.
9. Pacheco IL, Abril N, Morales-Prieto N, et al. *Fasciola hepatica* induces Foxp3 T cell, proinflammatory and regulatory cytokine overexpression in liver from infected sheep during early stages of infection. *Veterinary Research*. 2018;49(1):56. doi:10.1186/s13567-018-0550-x
10. Haçariz O, Sayers G, Flynn RJ, Lejeune A, Mulcahy G. IL-10 and TGF- β 1 are associated with variations in fluke burdens following experimental fasciolosis in sheep. *Parasite Immunology*. 2009;31(10):613–622. doi:10.1111/j.1365-3024.2009.01135.x
11. Mahmoud GS, Shakir MM. Globule leukocytes and mast cells in bile ducts of sheep with chronic fascioliasis. *The Iraqi Journal of Veterinary Medicine*. 1986;10(1):1–8.
12. Ruiz-Campillo MT, Molina-Hernández V, Bautista MJ, et al. *Fasciola hepatica* primo-infections and reinfections in sheep drive distinct Th1/Th2/Treg immune responses in liver and hepatic lymph node. *Veterinary Research*. 2023;54(1):2. doi:10.1186/s13567-022-01129-7
13. Zhang Y, et al. Metabolomic and systematic biochemical analysis of sheep infected with *Fasciola hepatica*. *Veterinary Parasitology*. 2022;310:109790.
14. Ferre I, Ortega-Mora LM, Rojo-Vázquez FA. Seroprevalence and plasma enzyme dynamics during chronic *Fasciola hepatica* infection in sheep. *Veterinary Parasitology*. 2003;112(3):201–212.
15. Sahibi H, et al. Immunodiagnosis of fascioliasis in ruminants by ELISA method: a mini-review. *Iranian Journal of Parasitology*. 2023;18(3):283–292.
16. Comparative analysis of anthelmintic treatments: impact on liver biomarkers and clinical recovery in sheep with fasciolosis. *Frontiers in Veterinary Science*. 2025;12:1520543.
17. Alvarez Rojas CA, Scheerlinck JP, Ansell BR, et al. Transcriptomic study on ovine immune responses to *Fasciola hepatica* infection. *PLOS Neglected Tropical Diseases*. 2016;10(8):e0005015.
18. Rodríguez E, Carasi P, Frigerio S, et al. *Fasciola hepatica* immune regulates CD11c⁺ cells by interacting with the macrophage Gal/GalNAc lectin. *Frontiers in Immunology*. 2017;8:264.
19. Kelley JM, Elliott TP, Beddoe T, Anderson G, Skuce P, Spithill TW. Current threat of triclabendazole resistance in *Fasciola hepatica*. *Trends in Parasitology*. 2016;32(6):458–469.
20. Sachdev D, Gough KC, Flynn RJ. The immune mechanisms of pathology during early infection of sheep with *Fasciola hepatica*. *Scientific Reports*. 2017;7:3160.