

Physiological, Biochemical, and Liver Enzyme Alterations in Albino Rats Experimentally Infected with *Echinococcus granulosus*

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Abstract

Objective: This study assessed physiological, biochemical, and liver enzyme alterations in rats experimentally infected with *Echinococcus granulosus* using two infection routes.

Methods: Fifty-four male rats were divided into three groups: orally infected with *E. granulosus* eggs, intraperitoneally inoculated with protoscoleces, and uninfected controls. Infection was assessed after four months using molecular methods. Serum ALT, AST, vitamin K2, lipid-profile parameters, and creatinine were measured using commercial assay kits.

Results: PCR amplification and PCR-RFLP analysis supported the presence of *E. granulosus* in infected animals. Triglyceride levels were significantly lower in both infected groups than in controls. ALT, LDL, and vitamin K2 were significantly higher in infected groups, while HDL was significantly elevated mainly in the orally infected group. AST, total cholesterol, and creatinine did not differ significantly among groups. Most correlations among biomarkers were weak to moderate.

Conclusion: Experimental *E. granulosus* infection was associated with alterations in selected lipid and liver-related biochemical markers in rats. The findings suggest possible route-dependent effects on lipid metabolism; however, further studies including histopathology, cyst-burden assessment, sequencing of representative tissue samples, and diagnostic accuracy analysis are required

Keywords: *Echinococcus granulosus*, PCR-RFLP, HinfI enzyme, physiological biomarkers, liver function enzymes, albino rat

Introduction

Cystic echinococcosis (CE), or hydatid disease, is a widespread zoonotic parasitic infection globally,¹ caused by the larval stage of *Echinococcus granulosus sensu lato*. The disease caused to be continued to be a severe health and economic concern, particularly in societies where livestock production and close contact between dogs and farm animals facilitate the transmission of the parasite.² Humans and numerous other mammals serve as intermediate hosts after accidental consumption of parasite embryonated egg shed in the feces of infected definitive hosts, especially dogs.³ Once ingested the released oncospheres enter the blood circulation, migrating to various sites in intermediate host's body, where they grow into hydatid cysts.⁴

Experimental animal models have been essential for investigating the pathophysiology, host-parasite interactions, and biological behavior of *Echinococcus granulosus*.⁵ Experimental infection using either with protoscoleces or parasites eggs of the parasite makes it possible to reproduce different stages of developmental of the hydatid cyst and to evaluate the clinical and biochemical changes occurring in host tissues.⁶ In addition to the physiological assessments, molecular identification has become an integral part of the research on hydatid disorders.⁷ DNA amplifying via PCR with restriction fragment length polymorphism (RFLP) analysis and PCR-RFLP are highly valuable molecular tools; PCR-RFLP in particular, is still a useful and economical approach to discriminate genetic differences by digesting amplified DNA fragments with restriction enzymes.⁸ To monitor these physiological abnormalities, has been extensively applied in experimental and clinical research, employing ELISA and other diagnostic methodologies.⁹ ELISA is based on the specific binding of antigens to

antibodies and provides sensitive measurements of biological molecules by enzyme-catalyzed colorimetric reactions.¹⁰

Cystic echinococcosis (CE) is an example of a zoonotic parasitic illness that highlights the complex affinity between animal hosts, environmental factors, and human infection.¹¹ Consequently, developing early detection methods and focused prevention measures for CE are of great scientific and public health concern. Frequent alterations in lipid metabolism are characteristic of dropped hepatic metabolic efficiency and parasite-induced host metabolic changes. Furthermore, elevated of liver enzymes is a consequence of damage to the hepatocyte, inflammation, and increased permeability of the membrane.¹² The hydatid infection may also affect the host lipid metabolism, as the parasite lacks the ability to synthesize de novo several key lipids and therefore depends on host-derived cholesterol, phospholipids, and lipoproteins for its growth and development. Therefore, changes in serum lipid profiles-including total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL)¹³ furthermore, factors such as the cyst's fertility status, and host type are related to various organic compound levels in cysts, including triglyceride, albumin, cholesterol, uric acid, glucose, creatinine, and total protein.¹⁴ In chronic liver disease, functional vitamin K insufficiency and coagulation abnormalities are often mediated by decreased availability of vitamin K through impaired bile secretion, decreased intestinal absorption of fat-soluble vitamins, and impaired hepatic metabolic activity.¹⁵

The majority of the experimental animals showed elevated liver enzymes simultaneously. The mechanism may involve inflammation and lesions in the liver, which limit the hepatic function and affect the production of vitamin K-dependent coagulation factors.¹⁶ Despite recent

advancements in diagnosing and studying cystic echinococcosis, significant gaps still exist in linking parasite infection with physiological changes and the molecular profile of the causative agent, *Echinococcus granulosus*. Thus, this investigation aimed to assess these physiological and molecular changes in experimental *Echinococcus granulosus*-infected rats by evaluating liver function test parameters, the lipid profile, levels of vitamin K2 and creatinine, and using molecular confirmation and characterization of the parasite infection by PCR-RFLP analysis with the *HinfI* enzyme.

Material and Methods

Experimental Design

Fifty-four healthy male albino rats (*Rattus norvegicus*), weighing 150–200 g, were acclimatized for seven days and then randomly allocated by computer-generated sequence into three groups of 18 animals each: Control, Egg-infected, and Protoscoleces-infected. All animals completed the four-month experiment, and no animals were excluded from analysis. The final number analyzed was 18 per group. Infection was assessed by PCR-based molecular methods at the end of the experiment. Blinding was not implemented during biochemical assays or statistical analysis. This is acknowledged as a limitation of the study.

Animal Ethics and Biosafety

All procedures involving dog infection, egg collection, oral rat infection, intraperitoneal rat infection, animal monitoring, infectious-waste handling, and zoonotic containment were approved by the Institutional Animal Care and Use Committee of Koya University, College of Science, Koya University (Approval No. 011Bio; December 17, 2024).

Animal Housing

Present research was performed as out in male albino rats (*Rattus norvegicus*) of 6–8 weeks old (150–250 g). Animals were kept in polypropylene cages under normal laboratory conditions ($22 \pm 2^\circ\text{C}$, 12 h light/12 h dark cycle). The approved protocol covered all experimental procedures, including dog infection, *Echinococcus granulosus* egg collection, experimental rat infection, animal handling, and zoonotic biosafety and containment measures. During the research period, rats had free access to regular laboratory pellet food and drinking water (*ad libitum*). Before experimentation, animals underwent a 7-days acclimatization period. All the procedures were followed ethical guidelines for animal research. One month after the usual course of treatment, the dog was experimentally infected by consumption with liver from a sheep with hydatid cysts with viable protoscoleces.

Anesthesia

The rats were anesthetized at the end of the experimental session with the combination of xylazine-ketamine (approximate ratio, 1:10) as has been utilized in previous study,¹⁷ by cardiac puncture from all groups. Serum was collected from blood samples by centrifugation, at 3,000 rpm for 15 min. The

serum was aliquoted and stored at -80°C for further physiological and liver function enzymes with biochemical examination.¹⁸ Animals were monitored daily for signs of pain or distress throughout the study. At the end of the 4-month experimental period, all rats were humanely euthanized under deep anesthesia before sample collection, in accordance with the approved animal ethics protocol.

Incidence of Infection

Fertile hydatid cysts harboring *Echinococcus granulosus* were acquired from sheep livers at the Koya municipal slaughterhouse in Erbil, Iraq. Protoscoleces (PSCs) from these cysts were used in the infection experiment. A healthy dog was treated with praziquantel, to eliminate Helminthes and establish a monospecific infection. The hydatid cysts were then infected liver fed to the dog for 3 consecutive days. One-month later eggs shed in the feces.

Each rat in the Egg group was housed individually during exposure and received 1000 *E. granulosus* eggs suspended in (100 mL) of drinking water. The suspension was freshly prepared, mixed immediately before administration, and provided in an individual bottle. Consumption was monitored until the full volume was ingested.¹⁹ Each rat was individually housed from the start to the final phase of the experiment so as to ensure that each animal received the prescribed infectious dose of *E. granulosus* eggs in the contaminated drinking water and to prevent cross-contamination within the animals.

Each rat in the Proto group received 2000 viable protoscoleces suspended in 400 μL sterile PBS by intraperitoneal injection. Protoscoleces viability was assessed using the eosin exclusion test. A total of 400 protoscoleces were counted, of which 396 were viable and 4 were non-viable, giving a viability rate of 99%. The viability % was computed (2000 protoscoleces).²⁰

$$\text{by the formula : Viability of protoscoleces (\%)} = \frac{\text{Number of viable protoscoleces}}{\text{Total number of Protoscoleces}} \times 100$$

Molecular Diagnosis

Genomic DNA was isolated from tissue using PrimePrep™ Genomic DNA Extraction Kit as per manufacturer specifications. The samples were kept at -20°C until PCR amplification. Specific Primers designed, established to amplify the gene fragment of *Cox3*. (5'-GTT TGG GGC TGG TTG GGT TA - 3') (5'-CAC GCC AAC ACA GTA CAA CG - 3'), as forward and reverse respectively, producing 450 bp amplicon. PCR performed in a 50 μL reaction with the following protocols: initial denaturation at 94°C for 5 minutes, subsequent to 30 cycles of denaturation at 94°C for 30 seconds, annealing at 63°C for 30 seconds, and extension at 72°C for 30 seconds, completing with final extension at 72°C for 5 minutes. Each 20 μL PCR reaction consisted of 10 μL of HotStarTaq DNA polymerase master mix buffer (Qiagen GmbH, Hilden, Germany), 1 μL of each primer, 5 μL of DNA template, and 3 μL of nuclease-free water. PCR products were separated via agarose gel electrophoresis on a 1.5% gel stained with ethidium bromide, operated at 85 V for 40 minutes, and subsequently detected with a UV transilluminator. Primer specificity was confirmed

by the successful amplification of a singular PCR product of the expected size (450 bp), and subsequent *Hinf*I digestion produced the expected restriction fragments of approximately 300 bp and 150 bp. The isolated PCR product of *cox3* gene predicted to restriction endonuclease enzyme of *Haemophilus influenza* (*Hinf*I).²¹

PCR-RFLP Procedure

The amplification of polymerase chain reaction (PCR) restriction fragment length polymorphism (PCR-RFLP) was conducted utilizing the *Hinf*I restriction enzyme (GeneCut™, Cat. No. GSR019). For each digesting procedure, 10 µL of PCR product, 3 µL of GeneCut buffer, 1 µL of *Hinf*I, and nuclease-free water were mixed to reach a complete quantity equal to 30 µL. The reaction mixture was prepared on ice and incubated at 37°C for 15–30 minutes, then undergoing inactivation of enzyme at 80°C for 20 minutes. The digested products were subjected to electrophoresis on a 1.5% (v/w) agarose gel containing ethidium bromide in 1× TBE solution at 100 V for 45 minutes, and the fragment sizes were measured using a 50-bp DNA ladder. Expected amplicon and fragment size approximately: Expected PCR amplicon size: primer size: 450–500 bp, *Hinf*I digestion site: GATC recognition site (according to RFLP manufactured instruction), (Biotech.).

Results

Viability of Protoscolex

Dye was rejected from living protoscolexes and they remained unstained, but non-viable organisms took up the dye and were red, as in Figure 1.

Polymerase Chain Reaction Amplification

DNA was extracted from various tissue samples and utilized as templates for PCR amplification of the mitochondrial *Cox3* gene, as confirmed by agarose gel amplification. The PCR analysis results obtained using the parasite sample were sequenced (Bioneer, Korea) and ultimately compared with other reference sequences for *E. granulosus* s.l. available in

GenBank utilizing the BLAST search. PCR results from egg of parasite positive sample were successfully sequenced and representative nucleotide sequence were deposited in the GenBank database with accession code PX754536, BLAST analysis showed that egg isolate belonged to *Echinococcus granulosus sensu lato*. Conventional PCR was performed individually on tissue samples acquired from each rat in each of the experimental groups. Each rat's multiple organ systems (liver, lung, kidney, brain, and spleen) were investigated individually. The organ mentioned in Table 1 indicates each PCR-positive tissue detected and does not represent a particular prevailing organ per animal; accordingly, multiple organs may be positive in the same rat. Percentages were determined using 18 rats as the denominator for each infected group ($n = 18$). All control animal tissue samples were tested using the same PCR method, yielding negative findings and confirming the absence of detectable *Echinococcus granulosus* DNA Figure 2.

PCR-RFLP

The *cox3* gene from tissue samples of both infected groups successfully amplified, yielding the expected PCR product on 1.5 % agarose gel. The expected amplicon size was approximately 450 bp. In silico digestion with *Hinf*I predicted fragments of (294 bp, 113 bp, and 46 bp). In the gel, the major visible fragments were approximately 300 bp and 150 bp. Minor fragments, if predicted, may not have been visible because of their small size and/or low staining intensity Figures 2 and 3.

Table 1. Distribution of PCR-positive organ samples among infected rats

Infected organ	Oral infection N. (%)	Intraperitoneal injection No. (%)
Liver	8 (44.44%)	9 (50.00%)
Lung	6 (33.33%)	5 (27.78%)
Kidney	2 (11.11%)	3 (16.67%)
Brain	1 (5.56%)	0 (0.00%)
Spleen	1 (5.56%)	1 (5.56%)

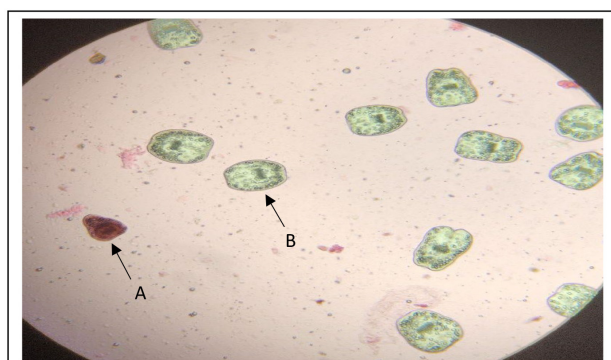


Fig. 1 Viability test of *E. granulosus* protoscolexes by microscopic examination: A. non-viable protoscolex. B. viable protoscolex (4x).

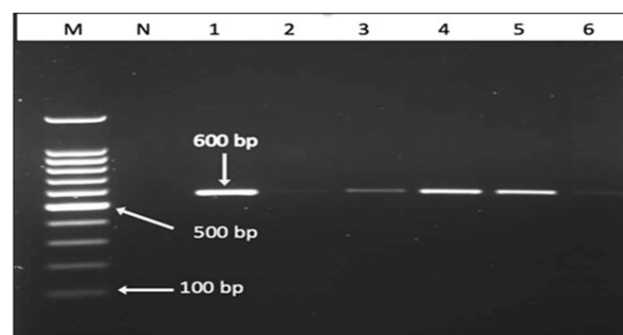


Fig. 2 Gel electrophoresis of the partially amplified of *Cox3* gene of *E. granulosus*. M = Ladder, N = negative control, 1 = Protoscolex, 2 = Brain sample, 3 = Egg from infected dog, 4 = Kidney sample, 5 = Lung sample, 6 = Liver sample.

ELISA Assays

ELISA/assay kits were obtained from ELK Biotechnology Co., Ltd. (Wuhan, China) and SunLong Biotech Co., Ltd. (China), as specified for each analyte.

Serum ALT and AST were measured as enzyme activity and reported in U/L according to the kit calibration. HDL, LDL, triglycerides, total cholesterol, creatinine, and vitamin K2 were measured using commercial kits according to the manufacturer's instructions. For diluted samples, final values were multiplied by the dilution factor before statistical analysis.

The raw data for all measured parameters and the comprehensive statistical outputs, including a variety of normality tests, Kruskal–Wallis tests, and correlation analyses, have been compiled and are available as supplementary material (or will be obtained from the corresponding author upon reasonable request, contingent upon the journal's policy). The data provided facilitates independent verification of the reported results. Principle information as in Table 2.

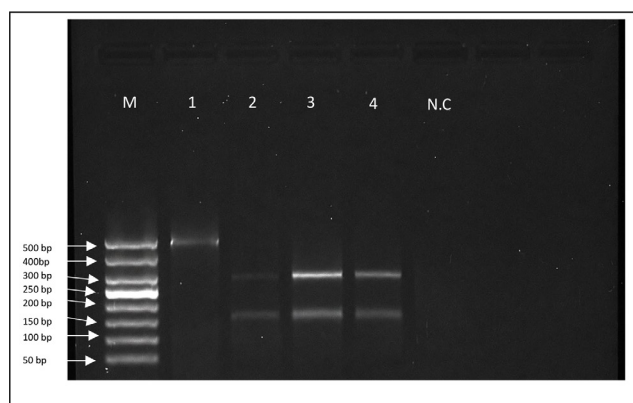


Fig. 3 PCR-RFLP analysis of the mitochondrial *cox3* amplicon of *Echinococcus granulosus*. Lane M: 50-bp DNA ladder; Lane 1: undigested PCR product; Lane 2: *HinfI*-digested egg-derived amplicon; Lane 3: *HinfI*-digested liver sample from egg-infected rat; Lane 4: *HinfI*-digested liver sample from protoscolex-infected rat; Lane NC: no-template negative control. Visible digestion bands were approximately 300 bp and 150 bp.

Statistical Analysis

Data were analyzed using SPSS version 25. Normality was assessed using the Shapiro–Wilk test. Normally distributed variables were summarized as mean $\pm$ standard deviation, while non-normally distributed variables were summarized as median and interquartile range. Differences among the three groups were assessed using the Kruskal–Wallis H test. When the Kruskal–Wallis test was significant, pairwise comparisons were performed using the Mann–Whitney U test with Bonferroni correction. The adjusted significance threshold for pairwise comparisons was $\alpha = 0.017$. Associations among biomarkers were evaluated using Spearman's rank correlation coefficient. A two-tailed *P*-value < 0.05 was considered statistically significant unless otherwise specified.

Biochemical Assays

Serum ALT and AST were quantified using commercially available ELISA kits according to the manufacturer's instructions. Although ALT and AST are commonly measured by conventional kinetic enzymatic activity assays in routine clinical chemistry, commercially available ELISA kits validated for rat serum were used in this study to provide standardized and sensitive quantification under identical assay conditions as the other serum biomarkers analyzed. The values obtained are given in U/L according to the calibration standards and instructions of the kit manufacturer. The units reported for each biomarker were those specified by the corresponding commercial assay kits and reflect the biological characteristics of the measured analytes. Vitamin K2 and creatinine were expressed in nmol/mL based on the kit calibration. Lipid biomarkers such as HDL, LDL, triglycerides and total cholesterol were expressed in $\mu\text{g/mL}$ according to the assay kits. The units of ALT and AST were U/L as these biomarkers are measures of enzyme activity. Therefore, the use of distinct measuring units reflects the analytical principles and calibration standards of each individual test, rather than differences in data reporting. Effect size for the Kruskal–Wallis test was estimated using eta squared ($\eta^2(H)$) according to the formula $\eta^2(H) = (H - k + 1) / (n - k)$, where *H* is the Kruskal–Wallis statistic, *k* is the number of groups (*k* = 3), and *n* is the total

Table 2. ELISA assay kits information

Parameters	Catalog. N	Detection range	Sensitivity	Intra-Assay	Inter-Assay	Sample D.	Manufacture
HDL	ELK1146	7.82–500 $\mu\text{g/mL}$	2.69 $\mu\text{g/mL}$	CV <8%	CV <10%	Not diluted	ELK, biotechnology
LDL	ELK7477	6.25–400 ng/mL	2.84 ng/mL	CV <8%	CV <10%	Not diluted	ELK, biotechnology
AST	ELK5636	0.79–50 U/L	0.33 U/L	CV <8%	CV <10%	Not diluted	ELK, biotechnology
ALT	ELK2683	0.79–50 U/L	0.26 U/L	CV <8%	CV <10%	Not diluted	ELK, biotechnology
Triglyceride	ELK8384	15.63–1000 $\mu\text{g/mL}$	1.6 $\mu\text{g/mL}$	CV <8%	CV <10%	Not diluted	ELK, biotechnology
Cholesterol	SL0162Ra	0.08–4 nmol/mL	0.01 nmol/mL	CV <10%	CV <12%	1:5	SunLog
Vitamin K2	SL1832Ra	26–1400 pg/mL	5.2 pg/mL	CV <10%	CV <12%	1:5	SunLog
Creatinine	SL0204Ra	1–70 nmol/mL	0.01 nmol/mL	CV <10%	CV <12%	1:5	SunLog

sample size ($n = 54$). Effect sizes were interpreted as small ($\eta^2 < 0.06$), moderate ($\eta^2 = 0.06-0.14$), and large ($\eta^2 > 0.14$).

The present parameters were compared among control, protoscolex-infected and egg-infected groups ($n = 18$ each) and significant changes in many biomarkers associated with hydatid infection were observed. Serum triglyceride levels were significantly distinct among the three groups ($P < 0.001$). The control group had the highest mean triglyceride concentration (86.50 ± 24.13), while both infected groups had significantly lower values, with the egg-infected group having the lowest (27.01 ± 10.44). Likewise, Serum HDL concentrations were significantly varied among the three groups (Kruskal–Wallis, $P < 0.001$). Pairwise comparison showed that the HDL level was considerably higher in the Egg group than in both the Control and Protoscolex groups (both $P < 0.001$) but no significant alternative was observed between the Protoscolex and Control groups ($P = 0.962$).

Differences among the groups were similarly significant in serum ALT activity ($P < 0.001$). The highest ALT level was detected in the protoscolex-infected group (0.93 ± 0.12), followed by the egg-infected group (0.88 ± 0.04), while the control group showed the lowest value (0.75 ± 0.10) suggesting possible liver-related biochemical alteration by experimental infection.

However, the total cholesterol did not reveal significant variations between the groups ($P = 0.238$). Numerically, the protoscolex-infected group exhibited a higher mean value (2.07 ± 0.30) than the control (1.85 ± 0.20) and egg-infected (1.67 ± 0.20) groups. The levels of vitamin K2 were significantly increased among the study groups ($P < 0.001$). The group infected with protoscolexes had the highest mean concentration (1451.32 ± 146.20), followed by the group infected with eggs (1337.76 ± 121.12), while the control group had the lowest concentration (1039.60 ± 94.52).

LDL values were also significantly different among groups ($P < 0.001$). The highest mean concentration of LDL was observed in the egg-infected group (7.27 ± 0.76), followed by the protoscolex-infected group (6.51 ± 0.66), and the lowest value was obtained in the control group (5.03 ± 0.67). Overall, these results showed that experimental infection with *Echinococcus granulosus* had substantial effect on triglyceride, HDL,

ALT, vitamin K2 and LDL concentrations, whereas total cholesterol did not alter. These data imply that hydatid infection produces changes in lipid metabolism and liver function in experimentally infected rats. The effect size analyses demonstrated large effect sizes for triglycerides ($\eta^2(H) = 0.662$), HDL ($\eta^2(H) = 0.633$), LDL ($\eta^2(H) = 0.495$), vitamin K2 ($\eta^2(H) = 0.391$) and ALT ($\eta^2(H) = 0.331$) indicating that experimental infection with *Echinococcus granulosus* significantly influenced these biomarkers. Conversely, total cholesterol had a low effect size ($\eta^2(H) = 0.002$), indicating that the experimental infection had little or no practical impact on blood cholesterol levels Table 3.

Median (IQR) of AST was 4.95 (3.48–53.91) in the control group, 9.43 (3.59–20.22) in the proto group, and in egg group 4.97 (3.54–22.83) in the egg group. The median AST value was numerically greater in the proto group than in the other groups, but the groups were not differed statistically ($P = 0.099$). The median creatinine readings in the control group were 31.25 (18.85–33.96), in the proto group 26.60 (22.97–41.24), and in the egg group 30.34 (29.02–31.65). There was no significant difference among the three groups ($P = 0.997$). These outcomes suggest that the experimental infection with *Echinococcus granulosus*, regardless of the method of infection was not significant in AST or creatinine levels compared to the control group. The analysis of effect size revealed that the effect of experimental infection on AST ($\eta^2(H) = 0.052$) and creatinine ($\eta^2(H) = 0.000$) was still negligible, which implies that the differences between the Control, Proto and Egg groups were low and of little practical significance Table 4.

Values are presented as median (IQR; 25th–75th percentile). P -values were calculated using the Kruskal–Wallis test. Statistical significance was defined as $P < 0.05$. Effect size is reported as $\eta^2(H)$.

Differences were calculated using pairwise analysis among the control, protoscolex-infected (Proto), and egg-infected (Egg) groups for each biochemical parameter. Serum triglyceride levels observed significant differences between the control and proto groups ($P < 0.001$) in addition to between the control and egg groups ($P < 0.001$). In contrast, there was no notable difference between the proto and egg groups ($P = 0.027$), revealing that both infection methods

Table 3. Parametric biochemical parameters in the study groups ($n = 18$ per group)

Parameters	Control (Mean $\pm$ SD)	Proto (Mean $\pm$ SD)	Egg (Mean $\pm$ SD)	P-value	Effect size $\eta^2(H)$
Triglyceride	86.50 $\pm$ 24.13	34.98 $\pm$ 9.82	27.01 $\pm$ 10.44	<0.001*	0.662
HDL	21.64 $\pm$ 0.25	21.19 $\pm$ 0.12	41.18 $\pm$ 21.21	<0.001*	0.633
ALT	0.75 $\pm$ 0.10	0.93 $\pm$ 0.12	0.88 $\pm$ 0.04	<0.001*	0.331
Cholesterol	1.85 $\pm$ 0.20	2.07 $\pm$ 0.30	1.67 $\pm$ 0.20	0.238	0.002
Vitamin K2	1039.60 $\pm$ 94.52	1451.32 $\pm$ 146.20	1337.76 $\pm$ 121.12	<0.001*	0.391
LDL	5.03 $\pm$ 0.67	6.51 $\pm$ 0.66	7.27 $\pm$ 0.76	<0.001*	0.495

Values are presented as mean $\pm$ SD. $P < 0.001$ was considered statistically significant (*).

Table 4. Non-parametric biochemical biomarkers in the study groups ($n = 18$ per group)

Parameter	Control median (IQR)	Proto median (IQR)	Egg median (IQR)	P-value	Effect size ($\eta^2(H)$)
AST	4.95 (3.48–53.91)	9.43 (3.59–20.22)	4.97 (3.54–22.83)	0.099	0.052
Creatinine	31.25 (18.85–33.96)	26.60 (22.97–41.24)	30.34 (29.02–31.65)	0.997	0.000

yielded in a significant decline in triglyceride levels as compared with the control group.

A marked absence of a significant change in HDL was observed between the control and proto groups ($P = 0.962$). The egg group had significantly elevated HDL levels compared to both the control ($P < 0.001$) and proto ($P < 0.001$) groups, indicating that oral infection specifically influenced HDL concentration. No substantial pairwise differences in AST were identified compared to the control, proto, and egg groups (control vs. proto, $P = 0.849$; control vs. egg, $P = 0.282$; proto vs. egg, $P = 0.164$), indicating comparable AST activity independent of the infection method. Serum ALT levels were significantly elevated in both infected groups compared to the control group (control vs. proto, $P < 0.001$; control vs. egg, $P < 0.001$). In contrast, there was no significant difference between the proto and egg groups ($P = 0.612$), and both infection methods induced comparable increases in ALT activity.

The non-existence of any significant effect on creatinine levels between the research groups (control vs. proto: $P = 0.924$; control vs. egg: $P = 0.975$; proto vs. egg: $P = 0.962$) indicates that the experimental infection did not impact renal function. In addition, total cholesterol levels demonstrated no significant differences amongst the three pairwise comparisons (control vs. proto, $P = 0.235$; control vs. egg, $P = 0.602$; proto vs. egg, $P = 0.217$), indicating that hydatid infection did not significantly impact total cholesterol concentrations. Serum vitamin K2 levels were substantially elevated in both infected groups compared to the control group (control vs. proto, $P < 0.001$; control vs. egg, $P < 0.001$), with no significant difference detected between the proto and egg groups ($P = 0.141$), suggesting a comparable effect of both infection methods on vitamin K2 concentrations. Eventually, LDL levels elevated significantly in both infected groups relative to the control group (control vs. proto, $P < 0.001$; control vs. egg, $P < 0.001$). Despite the omission of any significant effect between the proto and egg groups ($P = 0.775$), showing that both infection methods similarly affected LDL levels, a low significant difference was observed ($P = 0.027$). but this significance was lost after Bonferroni correction was used for multiple comparisons ($\alpha = 0.017$) as in Table 5.

Differences were established by using pairwise analysis among the control, protoscolex-infected (Proto), and egg-infected (Egg) groups for each biochemical parameter. Serum

triglyceride levels were considerably different between the control and proto groups ($p < 0.001$) and control and egg groups ($P < 0.001$). However, there was no significant distinction between the proto and egg groups ($P = 0.027$) suggesting that both modes of infection led to similar reduction in triglyceride levels when compared to the control group.

An absence of elevated a significant change in HDL was observed between the control and proto groups ($P = 0.962$). However, the egg group had significantly higher levels of HDL than both the control ($P < 0.001$) and proto ($P < 0.001$) groups, indicating that oral infection specifically affected HDL concentration. There were no significant pairwise differences in AST among the control, proto and egg groups (control vs. proto, $P = 0.849$; control vs. egg, $P = 0.282$; proto vs. egg, $P = 0.164$) indicating similar AST activity independent of the infection method. Serum ALT was significantly higher in the two infected groups than in the control group (control vs. proto, $P < 0.001$; control vs. egg, $P < 0.001$). However, there was no significant variation between the proto and egg groups ($P = 0.612$) and both modes of infection resulted in an equivalent rise in ALT activity.

Similarly, the absence of a significant effect on creatinine levels among the research groups (control vs. proto: $P = 0.924$; control vs. egg: $P = 0.975$; proto vs. egg: $P = 0.962$), indicates that experimental infection did not affect renal function. Similarly, total cholesterol was not significantly different in the three pairwise comparisons (control vs. proto, $P = 0.235$; control vs. egg, $P = 0.602$; proto vs. egg, $P = 0.217$), indicating that hydatid infection had no significant effect on the total cholesterol level. Serum vitamin K2 concentrations were significantly higher in both infected groups than in the control group (control vs. proto, $P < 0.001$; control vs. egg, $P < 0.001$), with no significant variation observed between proto and egg groups ($P = 0.141$), indicating a similar effect of both infection routes on vitamin K2 levels. Finally, LDL levels were considerably higher in both infected groups compared to the control group (control vs. proto, $P < 0.001$; control vs. egg, $P < 0.001$). Due to the lack any significant effect between the proto and egg groups ($P = 0.775$) recommending that both routes of infection had similar impact on LDL levels, A nominally significant difference has been found ($P = 0.027$), but this significance was lost after Bonferroni correction was used for multiple comparisons ($\alpha = 0.017$) as in Table 5.

Table 5. Pairwise comparisons across study groups

Parameter	(N = 18)			
	Control vs Proto	Control vs Egg	Proto vs Egg	Effect size ($\eta^2(H)$)
Triglyceride	<0.001***	<0.001***	0.027	0.662
HDL	0.962	<0.001***	<0.001***	0.633
AST	0.849	0.282	0.164	0.001
ALT	<0.001***	<0.001***	0.612	0.331
Creatinine	0.924	0.975	0.962	0.000
Cholesterol	0.235	0.602	0.217	0.002
K2	<0.001***	<0.001***	0.141	0.391
LDL	<0.001***	<0.001***	0.775	0.495

*Mann-Whitney U test with Bonferroni correction (adjusted $\alpha = 0.017$). ** $p < 0.001$. Effect sizes were derived from the overall Kruskal-Wallis test.

Pairwise comparisons revealed significant differences in triglyceride concentrations between the Control and Proto groups ($P < 0.001$) and between the Control and Egg groups ($P < 0.001$), in addition the difference between the Proto and Egg groups was not significant comparing to Bonferroni correction ($P = 0.027$). HDL levels were similar between the Control and Proto groups ($P = 0.962$), although indicated significant differences between the Control and Egg groups ($P < 0.001$) and between the Proto and Egg groups ($P < 0.001$). No significant pairwise differences were observed for AST, creatinine, or total cholesterol (all $P > 0.017$). ALT concentrations were significantly elevated in both infected groups as compared to the Control group (both $P < 0.001$), whereas no significant difference was identified between the Proto and Egg groups ($P = 0.612$). Furthermore, vitamin K2 and LDL levels showed significant difference between the Control group and both infected groups (both $P < 0.001$), while no significant differences were identified between the Proto and Egg groups ($P = 0.141$ and $P = 0.775$, respectively). Effect size estimates indicated large group effects for triglyceride ($\eta^2(H) = 0.662$), HDL ($\eta^2(H) = 0.633$), LDL ($\eta^2(H) = 0.495$), vitamin K2 ($\eta^2(H) = 0.391$), and ALT ($\eta^2(H) = 0.331$), whereas negligible effects were observed for AST ($\eta^2(H) = 0.001$), creatinine ($\eta^2(H) = 0.000$), and total cholesterol ($\eta^2(H) = 0.002$).

Spearman's rank correlation analysis was carried out to examine correlations among biochemical markers in the control group. The determined correlations ranged from weak to significant, including both positive and negative correlations. Triglycerides exhibited weak negatively correlated with HDL ($r = -0.341$), AST ($r = -0.059$), ALT ($r = -0.026$), vitamin K2 ($r = -0.184$), and LDL ($r = -0.217$), in addition to mild positive correlations with creatinine ($r = 0.418$) and total cholesterol ($r = 0.337$). HDL had a moderate positive correlation with AST ($r = 0.447$) and mild positive correlations with ALT ($r = 0.211$), creatinine ($r = 0.275$), and LDL ($r = 0.044$). Weak negative correlations were observed between HDL and total cholesterol ($r = -0.141$) as well as between HDL and vitamin K2 ($r = -0.328$). AST had a moderate positively correlated with ALT ($r = 0.402$), a weak positive correlation with creatinine ($r = 0.112$), and mild negative correlations with total cholesterol ($r = -0.131$), vitamin K2 ($r = -0.247$), and LDL ($r = -0.229$). ALT had a slight negative correlation with creatinine ($r = -0.345$) and a minor negative correlation with total cholesterol ($r = -0.010$). It had a weak positive correlation with vitamin K2 ($r = 0.052$) and LDL ($r = 0.325$). Creatinine had a moderate positive correlation with total cholesterol ($r = 0.317$), a moderate negative correlation with vitamin K2

($r = -0.559$, $P = 0.012$), and a weak negative correlation with LDL ($r = -0.226$). Vitamin K2 exhibited a weak positive correlation with total cholesterol ($r = 0.093$) and a weak negative correlation with LDL ($r = -0.349$). In the control group, most correlations among biomarkers were weak to moderate. Only the correlation between creatinine and vitamin K2 reached statistical significance, with creatinine and vitamin K2 showing $r = -0.559$ and $P = 0.012$, Table 6.

Discussion

Cystic echinococcosis (CE) is one of the most frequent zoonotic parasite-related infections around the worldwide, contributing to a significant public health and economic burden, especially within endemic areas where dogs, livestock, and humans are in close contact, facilitating the transmission of *Echinococcus granulosus*.^{1,21} Studies on host-parasite interactions and the physiological, biochemical and molecular changes that occur during infection require animal models. Therefore, in the present work, these changes were studied in an experimental rat model of *Echinococcus granulosus* infection induced via two alternative routes: intraperitoneal inoculation of live protoscoleces and oral administration of parasite eggs.

Successful establishment of experimental infection depends mostly on the vitality of the injected protoscoleces. Only protoscoleces showing a vitality of $\geq 99\%$, as indicated by the eosin red exclusion test, were used for intraperitoneal infection in the present investigation. The successful molecular identification and reproducible experimental infection require high protoscolex viability as only viable protoscoleces develop into the metacestode stage.^{11,22}

Molecular confirmation of infection was obtained by PCR-gel electrophoresis *cox3* gene amplifying, with HinfI-PCR-RFLP, which yielded two distinctive restriction fragments of approximately 300 bp and 150 bp in all positive samples. These results corroborate the presence of *Echinococcus granulosus* and highlight the usefulness of HinfI-PCR-RFLP as appropriate molecular technique to confirm the experimental infection. It has been demonstrated that PCR-RFLP using mitochondrial DNA markers can differentiate *Echinococcus* species and genotypes accurately, and it remains a rapid and cost-effective alternative to DNA amplifying by PCR and gel electrophoresis routine molecular diagnosis.^{23,24} Mitochondrial gene analysis has been identified as a valuable method for the characterization of amplified genes of *E. granulosus* isolates because of its high discriminating capacity and genetic stability.²⁵

Table 6. Spearman correlation matrix for control group

Variable	Trig	HDL	AST	ALT	Creatine	Chol	K2	LDL
Trig	1	-0.341	-0.059	-0.026	0.418	0.337	-0.184	-0.217
HDL	—	1	0.447	0.211	0.275	-0.141	-0.328	0.044
AST	—	—	1	0.402	0.112	-0.131	-0.247	-0.229
ALT	—	—	—	1	-0.345	-0.01	0.052	0.325
Creatinine	—	—	—	—	1	0.317	-0.559	-0.226
Chol	—	—	—	—	—	1	0.093	-0.349
K2	—	—	—	—	—	—	1	-0.015
LDL	—	—	—	—	—	—	—	1

Most previous studies on cystic echinococcosis have concentrated on lipid metabolism, inflammatory mediators, immunological responses and immune-related biomarkers, but relatively few have studied vitamin K2 metabolism together with liver enzymes and renal function markers in the same experimental model. Therefore, the present findings provide insight into a comparatively less investigated facet of host-parasite interactions by revealing changes in lipid metabolism, indicators of liver function and vitamin K2 after experimental *Echinococcus granulosus* infection.

Higher ALT in infected rats is in contrast with formerly reported clinical and experimental findings indicating liver damage related to hydatid infection. And correlates with earlier clinical and animal investigations, some studies indicated elevated AST, while others found no significant change depending on cyst location, stage and severity.²⁶ This study is consistent with Ramos et al, who found that the gradual expansion of hydatid cysts causes injury to the hepatic parenchyma and hence increasing serum transaminases Botezatu et al. also reported that elevated ALT levels were associated with hepatic involvement in cystic echinococcosis.²⁷

Serum cholesterol has been shown to be constant in certain investigations, despite considerable changes in other lipid fractions. Presented results are similar to that reported by Meerkhan and Mero et al. who similarly found little change in serum cholesterol in experimental hydatid infection. Other investigators, however, have documented lower cholesterol concentrations in chronic cystic echinococcosis, suggesting that protracted infection and a larger parasite biomass may increase cholesterol use by the parasite. Differences among studies might be due to host species, duration of infection, cyst burden and nutritional status.²⁸

Decreased triglyceride in both infected groups is consistent with parasite dependence on host lipids. Similar reductions in triglycerides were reported by Esteves et al. suggesting that *Echinococcus granulosus* has limited capacity for de novo lipid synthesis and depends on lipids received from the host.²⁹ The parasite takes up fatty acids and other necessary lipids from the host for its growth, membrane biogenesis and metabolic functions which may contribute in changes of the host lipid profile.³⁰

Serum HDL concentrations were considerably elevated only in the orally infected (Egg) group and no significant difference was observed between the Protoscolex and Control groups. This is consistent with that the route of infection influences host lipid metabolism. HDL is involved in reverse cholesterol transfer and possesses anti-inflammatory and anti-oxidant effects. In agreement with our results, Brunetti et al. showed that hepatic damage leads to dyslipidemia in cystic echinococcosis. Furthermore, *Echinococcus granulosus* utilizes host lipids for its proliferation and membrane biogenesis, which could explain the alterations observed in HDL metabolism.¹ Moreover, experimental data show that *Echinococcus granulosus* actively takes up host lipids by means of parasite lipoproteins, and thus may contribute to the alternation of host lipid homeostasis.³¹

Most studies have shown increasing levels of lipoprotein as reported in patients suffering from hydatid cyst infection in Saleem KA et al. which develop by elevating LDL as investigated in present study in both group of infection.³²

Recent assessments have also shown that renal involvement accounts for less than 5% of cystic echinococcosis

cases, and serum creatinine levels are generally unaffected in patients with uncomplicated hepatic disease. The present results indicate that experimental *E. granulosus* infection correlated with alteration for particular liver-related biochemical markers, particularly, ALT, while no significant differences in AST or creatinine were identified. These findings should be interpreted cautiously in the absence of histopathological confirmation, as assessed by Darani et al.³³

This is consistent with Shea and Booth et al. who pointed out the crucial activities of vitamin K apart from coagulation, such as the modulation of inflammation, oxidative stress and cellular metabolism.³⁴ Vitamin K2 is produced by gut microbiota and is involved in other physiological processes beyond coagulation, such as the modulation of inflammation, oxidative stress, and cellular metabolism. VK2 reduces the inflammatory response by decreasing the production of inflammatory cytokines (IL-4); VK-dependent proteins (VKDPs) represent another essential mechanism by which VK performs anti-inflammatory and immunomodulatory reactions. Recent reports have linked the medicinal values of VK with its anti-inflammatory activities.³⁵ In current findings Vitamin K2 levels were significantly elevated between both the orally infected (Egg) and intraperitoneally infected (Proto) groups compared to the Control group, however no significant difference was detected between the two infection methods. This finding indicates that the elevation of vitamin K2 may be linked to experimental *Echinococcus granulosus* infection rather than the mode of infection. To our knowledge, no previous studies have specifically investigated serum vitamin K2 concentrations in experimental or clinical cystic echinococcosis. The elevated vitamin K2 levels observed in this investigation should be considered an exploratory conclusion.

This study has several limitations. Histopathological examination was not performed, and cyst number, size, weight, and distribution were not quantified. Therefore, biochemical alterations could not be directly correlated with tissue pathology or infection burden. Biomarkers were measured at a single endpoint only. Diagnostic accuracy was not assessed. In addition, oral egg exposure may have introduced variation in individual parasite intake. Vitamin K2 findings should be considered exploratory and require confirmation in future studies.

Pairwise comparisons illustrated a significant difference in triglyceride, ALT, vitamin K2 and LDL levels between each infected group and control group. The difference relative to the proto and egg groups was statistically not significant. Conversely, the orally infected group exhibited an elevated level of HDL as compared to both control and the proto groups indicating that the method of infection may influence HDL metabolism.

Conclusion

The present study demonstrated biochemical alterations in rats experimentally exposed to *Echinococcus granulosus* through oral egg administration and intraperitoneal protoscolex inoculation. Infection was associated with decreased serum triglycerides and increased ALT, LDL, and vitamin K2, while AST, total cholesterol, and creatinine did not differ significantly. HDL increased mainly in orally infected rats, suggesting possible route-dependent effects on lipid metabolism. Molecular analysis supported parasite identification; however,

further studies incorporating histopathology, cyst-burden assessment, sequencing of representative tissue samples, and diagnostic accuracy analysis are required.³⁶

Conflicts of Interest Disclosure

None.

Data Availability

All raw data and complete statistical outputs supporting the interpretations of this research are available from the corresponding author upon reasonable request.

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