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# Indicative parameters for liver fascioliasis at pre-clinical and clinical phases in cows from Al-Diwaniyah city, Iraq

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**Abstract**

The current work emphasized understanding the liver functions while having pre-clinical or clinical fascioliasis accurately detect the disease phase from cows in Al-Diwaniyah City, Al-Qadisiyah Province, Iraq. The experimental design included the use of 30 cows in Al-Diwaniayh province divided into ten clinically healthy cows (control group), ten acutely infected cows with no apparent clinical signs (pre-clinical group), and ten chronically infected cows with observed clinical signs (clinical group), such as yellowish discoloration of the mucus membranes. Blood samples were collected from each cow for performing the following tests: Alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), serum levels of pyruvate, lactate, and Cholesterol. The results showed that all the enzymes from the pre-clinical group were significantly ($P<0.05$) higher in their serum levels compared with those from the control group to elevate to significantly ($P<0.05$) higher levels in the clinical group in comparisons with those from the control and pre-clinical groups, excluding the ALT that revealed no significant ($P>0.05$) difference between the pre-and clinical groups. A significant ($P<0.05$) increase was seen in the pre-clinical group compared to those from the control group, with no significant ($P>0.05$) difference between the pre-and clinical groups. No significant ($P>0.05$) differences were recorded between all study groups at serum pyruvate and lactate levels. The present study reveals that the fascioliasis, dependent on the infestation phase, can progressively change the serum levels of the parameters mentioned above and may feasibly be used together as indicators for the accurate detection of the disease stage.

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## Introduction

*Fasciola* parasites are of global significance, generating an illness in many animal species, including humans. *F. hepatica* causes a disease known as fascioliasis, a severe production inhibiting infection of ruminant cattle that induces extra cost of around $30 million of USD in the United Kingdom alone. This statistic is based on a rough calculation since the exact impact of fascioliasis on cow output is yet unknown. Fascioliasis cases and its geographic distribution have increased in many countries worldwide over the past decade, a development linked to global climate change and widespread animal movements. This tendency is expected to extend for the foreseeable future (1-6). Fascioliasis is more difficult to develop observed clinical signs in cattle than in small ruminants because the infection challenge from metacercariae must be more significant in cattle. These findings are attributed to their enormous liver, higher functioning capacity, and fibrous structure, unlike comparable animals. A subclinical chronic illness can be seen for fascioliasis in cattle, which is linked to liver destruction and blood loss due to parasites living in the bile ducts. Unlike sheep, cattle may acquire some degree of immunity over time. It also seems that infection risk rises with age, crediting that immunity does not preclude the emergence of new infections (7). Limited-clinical signs in fascioliasis in cattle are often ascribed to other factors, like as bad weather or undernutrition. Given the shortage of statistical data on output and parasite load, it has been challenging to evaluate the impact of subclinical disease to date (1). Infected metacercariae may be transmitted to cattle or various reasons, including lousy farm management. Previous research has indicated the importance of snail habitat occurrence on pasture, seasonal duration of pasture grazing, the percentage of grazed grass per diet, stocking rates, and the type for water supply, while herd size affects fluke infection risk assessment through unidentified or a mixture of pathways. Studies have discovered that these variables differ based on the environment and agricultural system in the locality (8,9). Fluke management should focus on snails and cattle to reduce infection rates. There has been little research on how grazing strategy may be combined with flukicides to reduce fluke. The application of molluscicides to reduce snail colonies is prohibited in some countries due to detrimental impacts on the environment. Another approach is to use pasture drainage. Regrettably, this is usually impracticable and costly, with harmful effects on the ecosystem (8).

Iraq is a known country for the occurrence of the disease (10,11); however, these studies focused on determining the presence of liver fluke as part of many parasitic infections of the liver. To accurately identify the disease phase using feasible tools, the current study was performed to differentiate between the pre-and clinical phases utilizing these tools.

## Materials and methods

### Animals and sampling

The experimental design included the use of 30 cows in Al-Diwanyiah province divided into ten clinically healthy cows (control group), ten acutely infected cows with no apparent clinical signs (pre-clinical group), and ten chronically infected cows with observed clinical signs (clinical group), such as yellowish discoloration of the mucus membranes. Before feeding time, blood samples were collected from the jugular vein in heparinized tubes.

### Blood analyses

The concentration of ALP was determined using the hydrolysis of n-nitrophenyl phosphate mentioned by Sepulveda (12). The level of GGT was detected employing a method adopted from Uçar *et al.* (13). The ALT level was evaluated using the Reitman-Frankel method by Crowley (14). The amount of Cholesterol was measured utilizing the Liebermann-Burchard test (15). The pyruvate and lactate were detected using the diphenylhydrazine method and paraoxydiphenyl reaction test, respectively (16).

### Statistical analysis

The GraphPad Prism v7 (GraphPad Inc., USA) was used to analyze and graph data based on Mean ± SEM. The null hypothesis was rejected if the *p* value was less than 0.05. A one-way ANOVA test was employed perform the study data processing.

## Results

The results showed that all the enzymes from the pre-clinical group were significantly (P<0.05) higher in their serum levels compared with those from the control group to elevate to significantly (P<0.05) higher levels in the clinical group in comparisons with those from the control and pre-clinical groups, excluding the ALT that revealed no significant (P>0.05) difference between the pre-and clinical groups. A significant (P<0.05) cholesterol increase was seen in the pre-clinical group compared to those from the control group, with no significant (P>0.05) difference between the pre- and clinical groups. No significant (P>0.05) differences were recorded between all study groups at serum pyruvate and lactate levels. Table 1 and figures 1-7 display the results in detail.

Table 1: Biochemical indicators of the blood serum of cows with fascioliasis

| Parameter | Serum concentration (Mean±SEM) | | |
|---|---|---|---|
| | Healthy animals n = 10 | Infected animals with the acute phase of fascioliasis n = 10 | Infected animals with chronic phase of fascioliasis n = 10 |
| ALP (d/ml) | 41.2±0.28 a | 80.3±0.31 b | 82.3±0.31 c |
| ALT (U/l) | 30.4±0.17 a | 32.9±0.22 b | 32.6±0.22 b |
| AST (U/l) | 39.3±0.16 a | 51.3±0.92 b | 55.3±0.92 c |
| GGT (U/l) | 32.2±0.13 a | 33.9±0.07 b | 35.9±0.07 c |
| Serum Pyruvate (µmol/l) | 127.40 ± 5.59 a | 120.80±5.18 a | 115.80±5.18 a |
| Serum Lactate (mmol/l) | 1.03 ± 0.15 a | 1.70±0.38 a | 1.30±0.38 a |
| Serum Cholesterol (mmol/l) | 5.15 ± 0.10 a | 6.92±0.18 b | 7.02±0.18 b |

Different letters mean significance (P<0.05) between groups.

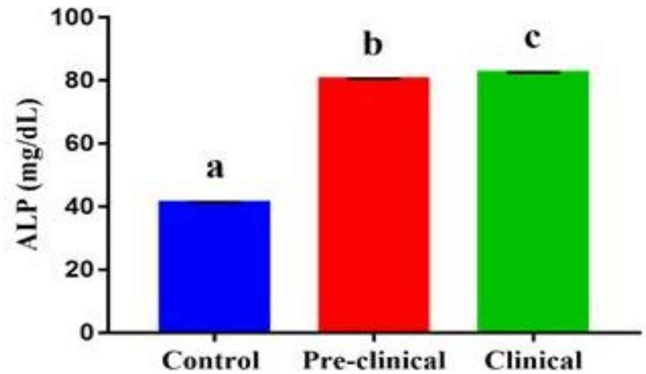


Figure 1: Serum concentration of alkaline phosphatase (ALP) from cows with pre-clinical and clinical fascioliasis. Different letters mean significance (P<0.05) between groups.

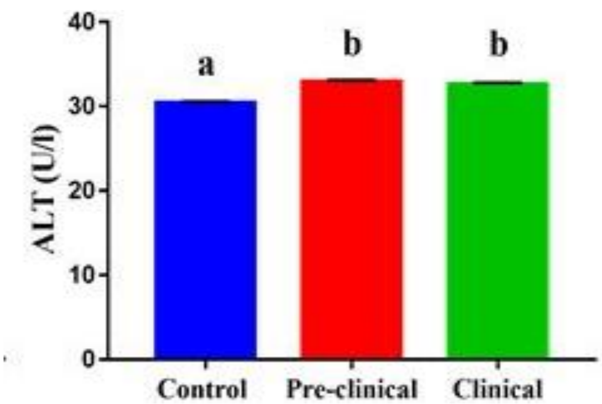


Figure 2: Serum concentration of alanine transaminase (ALT) from cows with the pre-clinical and clinical fascioliasis. Different letters mean significance (P<0.05) between groups.

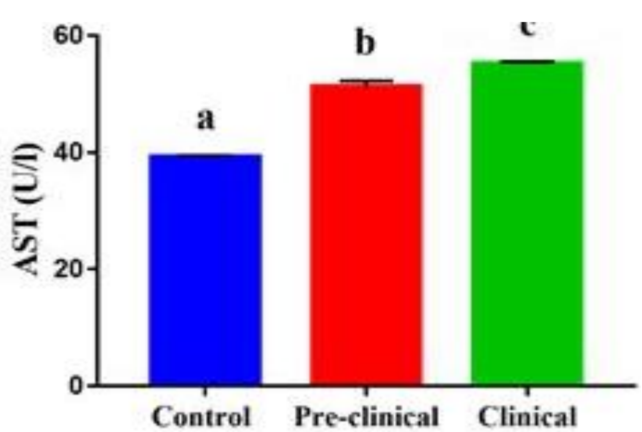


Figure 3: Serum concentration of aspartate transaminase (AST) from cows with the pre-clinical and clinical fascioliasis. Different letters mean significance (P<0.05) between groups.

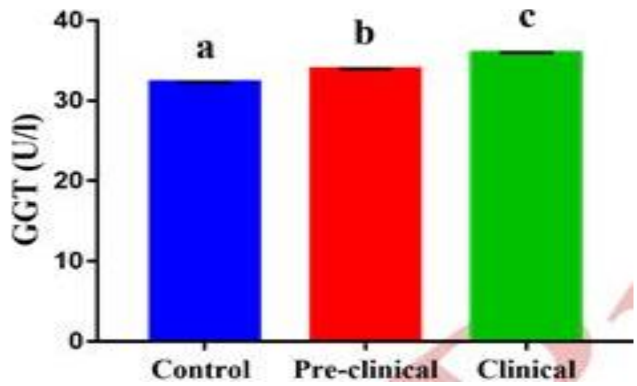


Figure 4: Serum concentration of gamma-glutamyl transferase (GGT) from cows with the pre-clinical and clinical fascioliasis. Different letters mean significance (P<0.05) between groups.

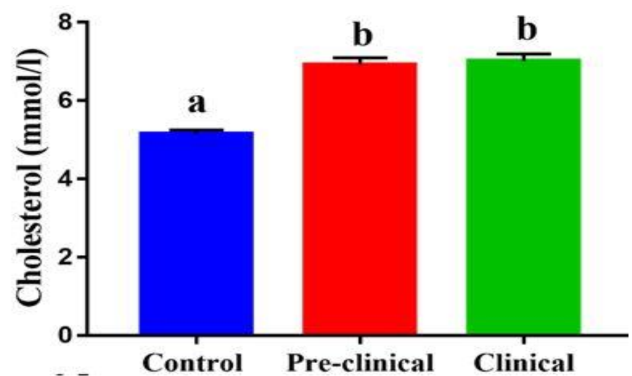


Figure 5: Serum concentration of Cholesterol from cows with the pre-clinical and clinical fascioliasis. Different letters mean significance (P<0.05) between groups.

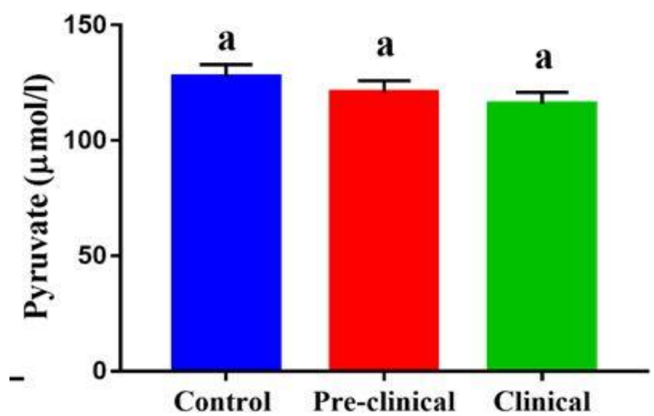


Figure 6: Serum concentration of pyruvate from cows with the pre-clinical and clinical fascioliasis. Different letters mean significance (P<0.05) between groups.

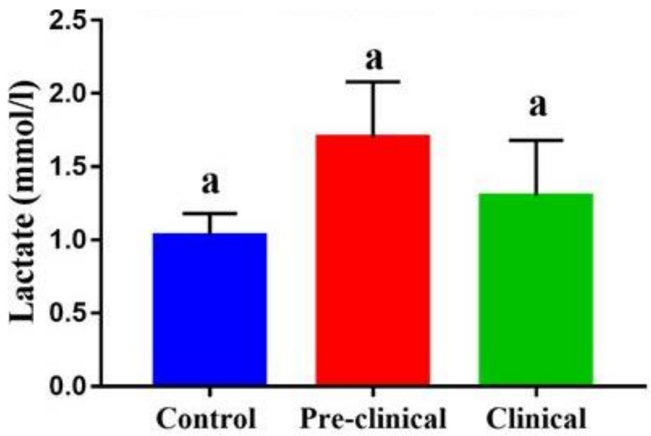


Figure 7: Serum concentration of lactate from cows with the pre-clinical and clinical fascioliasis. Different letters mean significance (P<0.05) between groups.

## Discussion

The liver fluke in cows can go undiagnosed on almost all occasions. This is highly true when many cases of bovine fascioliasis can only be detected in abattoirs. This phase can go from acute to chronic form without clear clinical signs, causing a subclinical infection (17). Following easy, feasible, and cost-effective methods for diagnosing the disease phases is essential for healthy animals and the economy. For this reason, the present work was conducted.

Nasreldin and Zaki (18) have found that 38 (16.81%) of 226 cattle were detected having *F. hepatica* after performing the postmortem examination (PME). They reported that serum alanine aminotransferase, glutamate dehydrogenase, aspartate aminotransferase, and γ-glutamyl transferase were highly increased due to the infection. They documented that ALT was 30.85 U/L, AST was 100.44 U/L, and GGT was 57.22 U/L in the infected group. These animals were confirmed for the diagnosis only after they were subjected to PME, indicating that they did not show observable signs to perform an early clinical diagnosis (18). The present work results agree with those by Nasreldin and Zaki (18), in which the ALT levels are comparable from the present study and that by Nasreldin and Zaki (18). However, the AST results were lower from the current work (51U/l) than Nasreldin and Zaki's (18). For the GGT, the levels in the present work were (33.9U/l), which is lower than that of Nasreldin and Zaki (18). These variations could be attributed to maybe lower numbers of migrating and adult parasites present in the liver and the bile ducts of the infected animals from the current investigation. This can oppositely be presented in the clinical phase, in which higher parameter reads were recorded, probably due to long term infection with low numbers of parasites or due to infection with high numbers of these trematodes during a short period.

Our findings are in agreement with those documented by Jarujareet *et al.* (19) and Kowalczyk *et al.* (20), who explained that the elevated serum levels of ALT and AST are caused by the inflammatory process that occurs in the hepatic tissues with the presence of the ongoing destructions by the migration of the fluke that results in the elevated enzymatic levels in the sera of the infected animals. Moreover, the migratory process enhances the release of the reactive oxygen species, which leads to more liver damage. The increases in serum GGT could be due to the adult parasites' destruction of the bile duct, which causes hyperplastic cholangitis (21-25).

## Conclusion

The present study reveals that the fascioliasis, dependent on the infestation phase, can progressively change the serum levels of the parameters mentioned above and may feasibly be used together as indicators for the accurate detection of the disease stage.


## Acknowledgments

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## Conflict of interests

The authors have not received any funding or benefits from the industry, the agency of financing, or elsewhere to conduct this study.

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# المؤشرات الإرشادية لداء المتورقات الكبدية في مرحلتي ما قبل السريرية والسريرية في الأبقار من مدينة الديوانية، العراق

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**الخلاصة**

تم إجراء هذا العمل الحالي لغرض فهم وظائف الكبد أثناء الإصابة بالطور ما قبل السريري أو الطور السريري لداء المتورقات لغرض التشخيص الدقيق للطور المرضي في أبقار في مدينة الديوانية، محافظة القادسية، العراق. تضمن التصميم التجريبي استخدام ٣٠ بقرة في مزرعة مقسمة إلى ١٠ أبقار غير مصابة سريريًا (مجموعة السيطرة)، وعشرة أبقار مصابة بشكل حاد مع عدم وجود علامات سريرية واضحة (المجموعة ماقبل السريري)، و ١٠ أبقار مصابة بشكل مزمن مع وجود علامات سريرية ملحوظة (المجموعة السريرية)، مثل تلون الأغشية المخاطية باللون الأصفر. تم جمع عينات الدم من كل بقرة لإجراء الفحوصات التالية: الفوسفاتيز القلوية، ناقلة أمين الألانين، ناقلة أمين الأسبارتات، ناقلة الببتيد غاما غلوتاميل، حامض البيروفيت، حامض اللاكتيت، والكوليسترول. أظهرت النتائج أن تركيز جميع الإنزيمات من المجموعة قبل السريري كانت أعلى بشكل ملحوظ مع تلك التراكيز الموجودة في مجموعة السيطرة لترتفع إلى مستويات أعلى بشكل ملحوظ في المجموعة السريرية بالمقارنة مع تلك المستويات في مجموعتي السيطرة وقبل السريري، باستثناء ناقلة أمين الالنين الذي أظهر عدم وجود فرق معنوي بين مجموعتي السريرية وقبل السريري. بالنسبة للكوليسترول، لوحظت زيادات معنوية في تراكيز المجموعة قبل السريري مقارنة بتلك التراكيز من مجموعة السيطرة مع عدم وجود فرق معنوي بين مجموعتي السريرية وقبل السريري. لم تسجل الدراسة فروق معنوية في مستويات الحامض البيروفي وحامض اللاكتيت بين جميع مجموعات الدراسة. تكشف الدراسة الحالية أن داء المتورقات واعتمادا على طور الإصابة يمكن أن يغير تدريجياً في تراكيز الاختبارات المذكورة أعلاه ويمكن استخدامها معًا كمؤشرات للكشف الدقيق عن طور المرض.