

## Some Physiological and Pathological Study of Fibrinous Pericarditis and Perihepatitis in Chickens Exposure to Airsacculitis

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DOI: [10.23975/bjvetr.2023.179944](https://doi.org/10.23975/bjvetr.2023.179944)

Received: 19 June 2023 Accepted: 29 June 2023.

### Abstract

The current study was conducted to detect fibrinous pericarditis and perihepatitis in naturally infected broilers and layers chickens in Basrah province, Iraq, on the basis of clinicopathological findings, and blood alterations. Suspected samples were obtained from several locations in Basrah province (Al-Hartha, Abo Alkaseb, and Al-Qurnah). The result of this study showed some birds presented clinical symptoms like the chicken reduced feed efficiency, weight loss, ruffled feathers, labored rapid breathing, coughing, and reduced consciousness. The blood tests revealed a significant increase ( $P \leq 0.05$ ) in WBCs, heterophils, and lymphocytes. The results of biochemical revealed a significant increase ( $P \leq 0.05$ ) in (ALT and AST and troponin titer) compared with healthy birds. The macroscopical of the internal organs showed severe accumulation of white typical fibrinous exudate in the pericardial sac. In addition, there is an enlargement of the liver with obvious of white typical fibrinous exudate in the parietal surface of the liver. The histopathological section of the heart showed severe infiltration of inflammatory cells in the pericardial sac with fibrinous exudation. There is an edematous exudate in the myocardial muscle fibers. The histopathological section of the liver showed thick severe fibrinous exudate capsulated on the liver surface. The histochemical section of the heart and the liver showed bluish-positive staining of collagen and fibrin fibers that accumulate in the pericardial sac and in the hepatic capsular. The study concluded that fibrinous pericarditis and perihepatitis lead to an effect on the health status and performance of different species of chickens as a broiler or layer.

**Keywords:** Hematology, Biochemical, Histopathology, Chickens.

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## Introduction

Fibrinous pericarditis and perihepatitis in poultry cause considerable economic and welfare problems in chickens. Its frequent occurrence caused adverse effects on growth and health. Clinically, the disease is named airsacculitis or chronic respiratory disease. It has respiratory signs, growth retardation, reduced feed intake, and increased mortality (1).

Fibrinous pericarditis, perihepatitis, and serositis secondary to *Mycoplasma* or *Escherichia coli* infection chicken. These systemic bacterial infections may have one or several bacterial causative agents (2). Colibacillosis is most commonly seen with other diseases (Mycoplasmosis or chronic respiratory disease (CRD)), which causes a combined infection known as complicated chronic respiratory disease (3). According to (4), the subacute form of chicken colibacillosis is characterized by fibrinous pericarditis and fibrinous perihepatitis. The acute form is marked by septicemia that results in mortality.

In addition, *Mycoplasma gallisepticum* infections in chickens cause relatively mild catarrhal sinusitis, tracheitis, and airsacculitis (5). Inflammatory cells, fibrin, and fluid are released when bacteria cause damage to the blood vessel (6).

The current study aimed to increase knowledge of the relative severity of lesions is necessary to establish a meaningful definition of susceptibility to colibacillosis, and the association between lesions. Therefore, the screening and detection of fibrinous pericarditis and fibrinous

perihepatitis as well as hematological study and biochemical analysis to evaluate the heart and liver function tests. In addition, macroscopically identification of the main pathognomonic gross lesions of the fibrinous pericarditis and perihepatitis as well as microscopically screening of the characteristic lesions of fibrinous pericarditis and perihepatitis. Furthermore, histochemical identification of fibrin deposition by using special stains.

## Materials & Methods

### **Clinical history, Gross lesions, Total leukocyte counts, biochemical analyses and histopathologic examination.**

The study focused on clinical symptoms, post mortem changes, total leukocyte counts, biochemical analyses, and histopathological changes. The duration of the present study was from October 2022 to March 2023. The samples were obtained at random from several locations in Basrah province (Al-Hartha, Abo Alkaseb, and Al-Qurnah) based on clinical suspicion for the diagnosis. All samples were presented to the University of Basra's Department of Veterinary Pathology and Poultry Diseases for necropsy evaluation. All cases were investigated for visceral gross lesions. The blood samples were obtained via brachial vein puncture using a new single-use syringe. To analyze WBC counts and perform various heart and liver function tests, samples were maintained in two separate test tubes (7), (8). The preparation of postmortem lesion was processed depending on the following steps (9).

There are 75 samples in total for the investigation, 50 out of 75 examined birds exhibited different degrees of fibrinous pericarditis and fibrinous perihepatitis. Normal birds were saved as control. Liver function analysis for the measurement of alanine transaminase and aspartate aminotransferase according to (10), Heart function analysis for the measurement of troponin titer according to (2). Heart and liver samples were preserved in formalin (10%) for 72 hours, processed according to the routine procedure, sectioned at 5 microns and stained with Haematoxylin & Eosin according to (11), (12) and stained with special dye of Mallory's trichrome stain according to (13) and examined under a light microscope depending on (14).

## Results

### **Clinical history, Gross lesions, Total leukocyte counts, biochemical analyses and histopathologic examination.**

In the present study, a total of 75 broilers and layers were clinically examined. The clinical signs observed where the chicken may put the beak into the litter to support the head as they squat with their eyes closed. In addition, weight loss, depression, and ruffled feathers. Furthermore, labored rapid breathing with, coughing, abdominal distention, reduced consciousness, reduced feed efficiency, decreased water consumption, and decreased growth. The clinical symptoms mostly appeared in chickens with 3 to 4 weeks of age. The results indicated that the infected chicken

with fibrinous pericarditis and perihepatitis in the broiler was higher than the layer with a percentage of infection 35 (70%) and 15 (60%), respectively (Table 1).

### **Hematological Results:**

#### **Differential WBCs count:**

There is a significant ( $P \leq 0.05$ ), however, increase in WBCs in the infected group compared to the control group, which revealed ( $12.58 \pm 0.54$ ) of fibrinous pericarditis and perihepatitis group and ( $10.7 \pm 0.17$ ) in the control group, (Table 2). In addition, the heterophil level count revealed a significant increase ( $P \leq 0.05$ ) in the infected group compared with the control group, with ( $59.1 \pm 1.87$ ) of the infected group and ( $28.68 \pm 1.16$ ) of the control group, (Table 2).

The infected groups lymphocyte level count increased significantly ( $P \leq 0.05$ ) and exhibited a value of ( $34.8 \pm 1.49$ ) compared with the control group's value of ( $29 \pm 0.44$ ), (Table 2).

The results of the Eosinophils, Basophils, and Monocytes level count also revealed that there was no difference ( $P \leq 0.05$ ) between the infected group and the control group.

#### **Biochemical analysis:**

The biochemical study's results noticed a significant increase ( $P \leq 0.05$ ) in the concentrations of alanin transaminase (ALT), aspartate *aminotransferase* (AST) and troponin (Tn), which showed ( $52.33 \pm 5.03$ ), ( $65.6 \pm 0.5$ ) and ( $0.8 \pm 0.16$ ) in fibrinous pericarditis and perihepatitis group respectively compared with control group (Table 3).

**Table (1): Number and percentage of infected with fibrinous pericarditis and perihepatitis in broiler and layer**

Birds	NO. of Birds	NO. of infected birds	% of infected
Broilers	50	35	70%
Layers	25	15	60%

**Table (2): Hematological result of fibrinous pericarditis and perihepatitis in both infected and control birds.**

Parameter	Control chickens	Infected chickens
WBC ( $10^9/L$ )	10.7±0.17	12.58±0.54*
Heterophils %	28.68±1.16	59.1±1.87*
Lymphocyte%	29±0.44	34.8±1.49*
Eosinophil %	1.76±0.23	1.5±0.16
Basophils %	0.11±0.01	0.08±0.02
Monocyte %	1.52±0.12	1.28±0.09

\* Results are expressed as Means and S.E of mean, significant at ( $P \leq 0.05$ )

**Table (3): Biochemical result of fibrinous pericarditis and perihepatitis in both infected and control birds**

Parameters	Control	Infected
ALT (U/L)	28.6±1.61	52.33±5.03*
AST (U/L)	26.4±1.2	65.6±0.5*
Tn (ng/ml)	0.21±0.18	0.8±0.16*

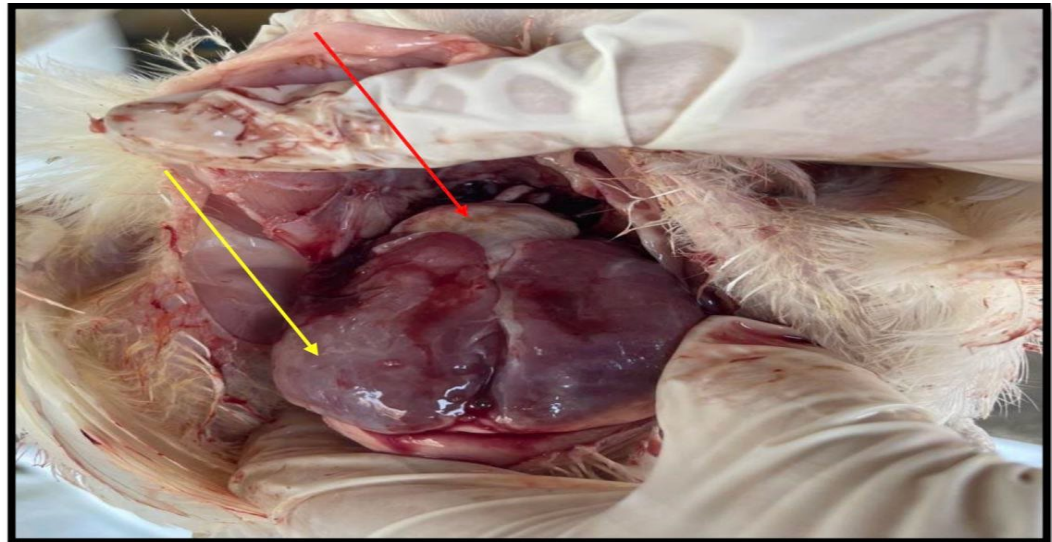
\* Results are expressed as Mean and S.E of mean, significant at ( $P \leq 0.05$ )

## Pathological results

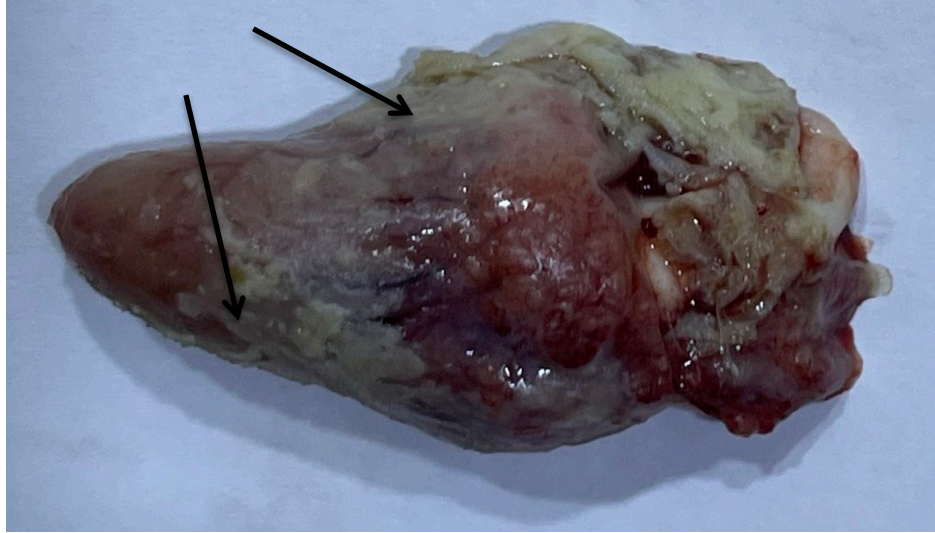
### Macroscopical results:

The Macroscopical results of the internal organs of the infected chicken showed severe accumulation of white typical fibrinous exudate in the pericardial sac referring to severe fibrinous pericarditis. In addition, there is an enlargement of the congested liver (hepatomegaly) with obvious of white typical fibrinous exudate in the parietal surface of the liver referring to fibrinous perihepatitis Figure (1). The heart

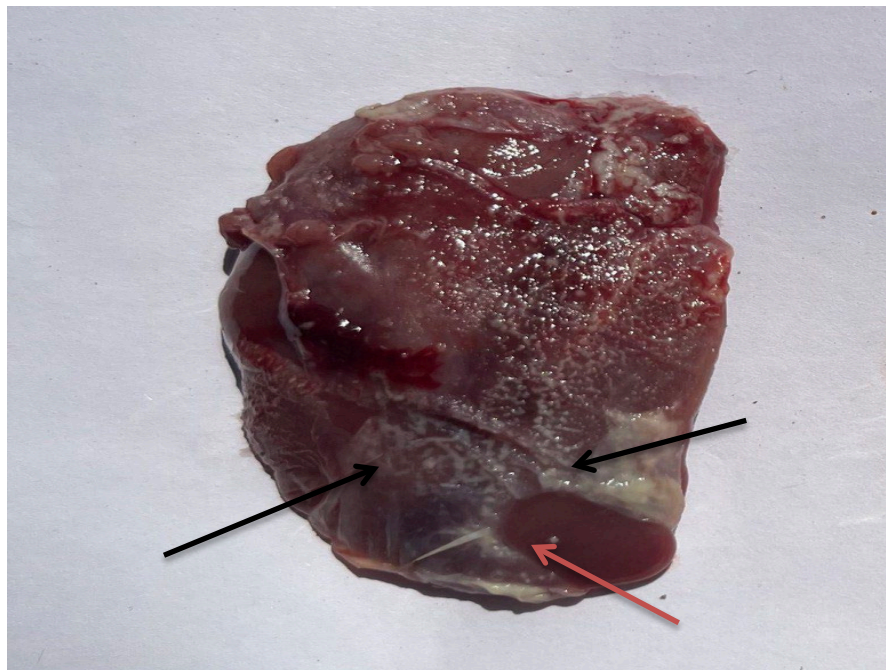
of the infected chicken showed severe accumulation of white typical fibrinous exudate in the pericardial sac referring to fibrinous pericarditis Figure (2). The liver of the infected chicken showed severe accumulation of white typical fibrinous exudate in the liver surface referring to fibrinous perihepatitis, also there is an enlargement of the liver referring to hepatomegaly Figure (3).



**Figure (1):** Macroscopical appearance of the internal organs of the diseased chicken showed severe accumulation of white typical fibrinous exudate in the pericardial sac referring to severe fibrinous pericarditis (red arrow). In addition, there is an enlargement of the congested liver (hepatomegaly) with obvious of white typical fibrinous exudate in the parietal surface of the liver referring to fibrinous perihepatitis (arrow in yellow).



**Figure (2):** Gross appearance of the heart of the infected chicken showed severe accumulation of white typical fibrinous exudate in the pericardial sac referring to fibrinous pericarditis (black arrow).



**Figure (3):** Gross appearance of the liver of the infected chicken showed severe accumulation of white typical fibrinous exudate in the liver surface referring to severe fibrinous perihepatitis (black arrow). In addition, also there is an enlargement of the liver (red arrow).

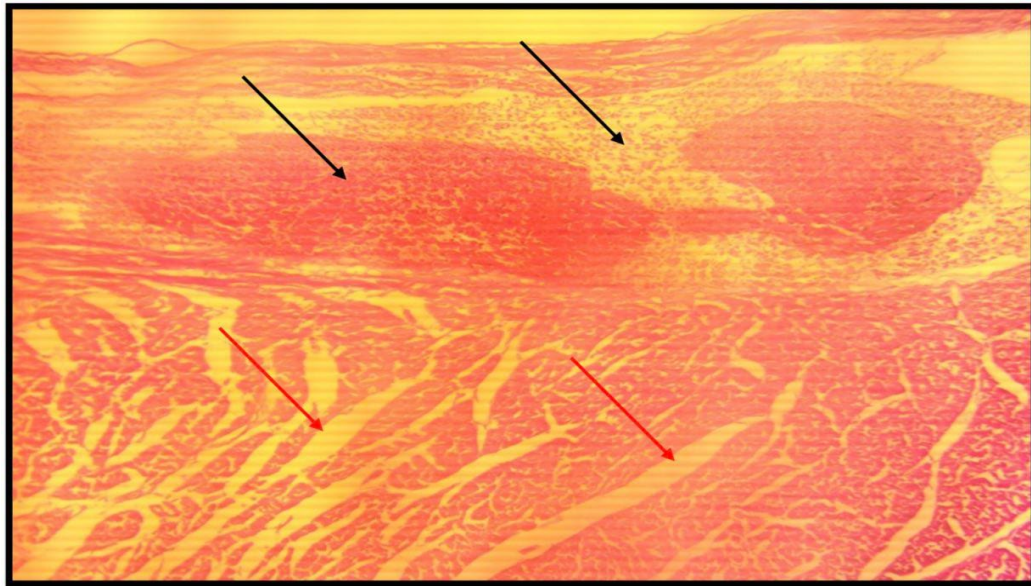


### Microscopical results:

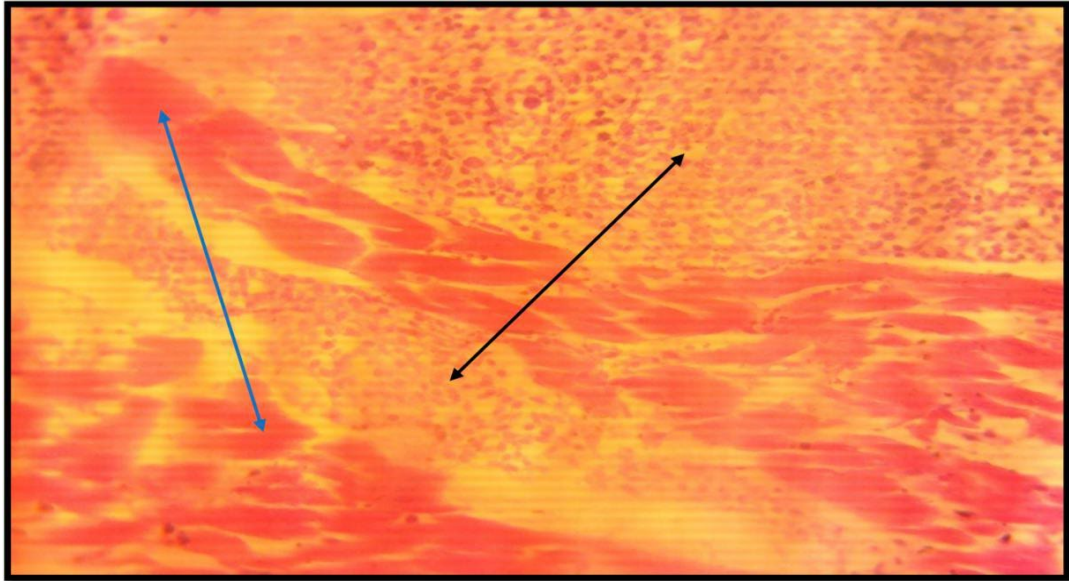
The histopathological section of the heart of infected chicken showed severe infiltration of inflammatory cells in the pericardial sac with fibrinous. In addition, there is an edematous exudate in the myocardial muscle fibers as in Figure (4). Another histopathological section of the heart of infected chicken showed severe infiltration of polymorphonuclear inflammatory cells in the area between the pericardium and the myocardial muscle fibers. In addition, there

was necrotic foci of myocardial muscle fibers section Figure (5).

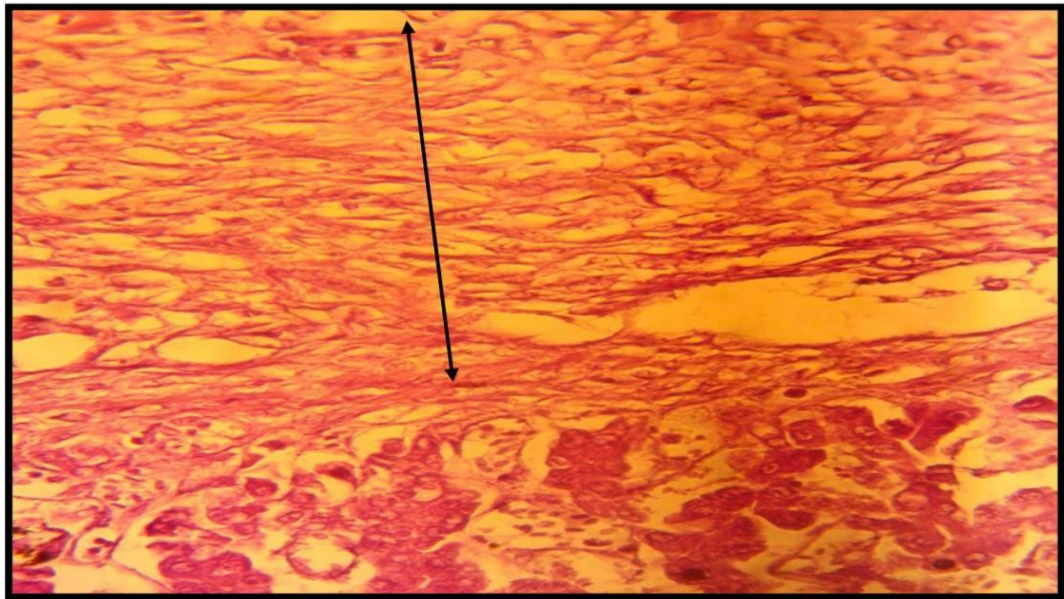
The Histopathological section of the liver of infected chicken showed thick severe fibrinous exudate capsulated on the liver surface Figure (6). Another histopathological section of the liver of infected chicken showed multiple micro-abscesses in the hepatic parenchyma, also dilation of sinusoids Figure (7).



**Figure (4):** Histopathological micrograph of the heart of infected chicken showing severe infiltration of inflammatory cells in the pericardial sac with fibrinous exudation (black arrows) In addition, there is an edematous exudate in the myocardial muscle fibers (red arrows). H&E stain. 100X.

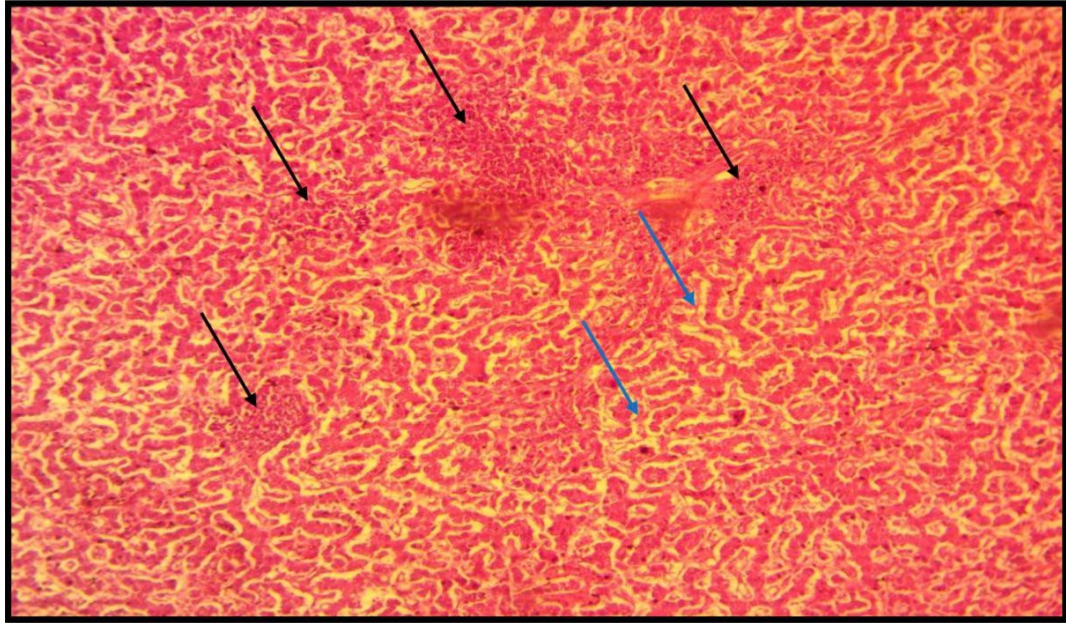


**Figure (5):** Histopathological micrograph of the heart of infected chicken showing severe infiltration of polymorphonuclear inflammatory cells in the area between the pericardium and the myocardial muscle fibers (double-headed black arrow). In addition, there are necrotic foci of myocardial muscle fibers (double-headed blue arrow). H&E stain. 400X



**Figure (6):** Histopathological micrograph of the liver of infected chicken showing thick severe fibrinous exudate capsulated on the liver surface (Black arrows with two heads). H&E stain at 400X



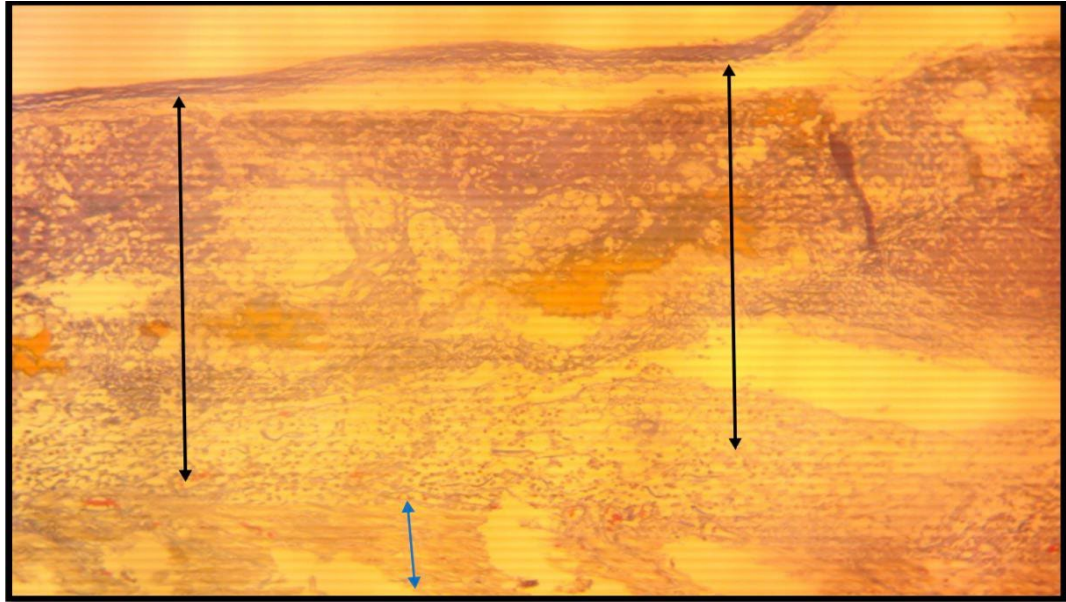


**Figure (7): Histopathological micrograph of diseased chicken liver showing numerous micro-abscesses in the hepatic parenchyma (black arrows) and sinusoidal dilatation (blue arrows), 100X H&E stain.**

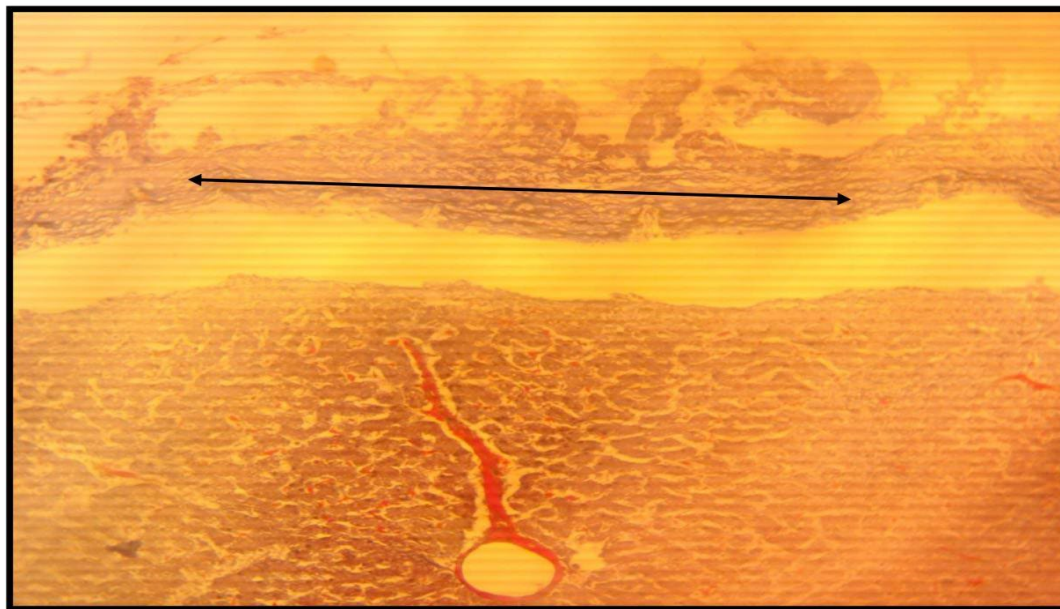
### **Histochemical Results**

A histochemical section of the heart showed bluish positive staining of collagen and fibrin fibers that extensively accumulate in the pericardial sac referring to active fibrinous pericarditis. While the myocardial muscle fibers appeared in yellowish color in

Mallory's trichrome stain (Figure 8). A histochemical section of the infected liver showed bluish positive staining of collagen and fibrin fibers that extensively accumulate in the hepatic capsular region referring to active fibrinous perihepatitis in Mallory's trichrome stain (Figure 9).



**Figure (8):** Histochemical micrograph of the heart of the infected chicken showing bluish positive staining of collagen and fibrin fibers that extensively accumulate in the pericardial sac referring to active fibrinous pericarditis (double-headed black arrows). While the myocardial muscle fibers appeared in yellowish color (double-headed blue arrow). Mallory's trichrome stain. 100X.



**Figure (9):** Histochemical micrograph of the liver of the infected chicken showing bluish positive staining of collagen and fibrin fibers that extensively accumulate in the hepatic capsular region referring to active fibrinous perihepatitis (double-headed black arrow). Mallory's trichrome stain. 100X

## Discussion

The clinical signs were reported in the current study in chickens represented by reduced feed efficiency due to weight loss and decreased growth, in addition to many cases chickens, may hunch their bodies. While sitting with their eyes closed and placing their beaks in the litter to support their heads, abdominal distention, reduced consciousness, depression, and ruffled feathers, as well as labored rapid breathing with and coughing. These signs occur as a result of the presence of bacteria when bacteria release endotoxin in the blood because of the inability of birds to eat and maybe an inability to focus, close their eyes, or cause rapid breathing. These clinical signs were also noted by (15, 16,17).

The current study indicated a significant rise in total leukocyte count, which might be explained by an increase in heterophils and Lymphocytes. There is evidence that acute inflammatory conditions, especially those caused by bacterial infections are related to an increase in total white blood cell count. This finding might be due to infectious agents and tissue damage products cause a variety of cells to produce growth factors, cytokines, and other inflammatory mediators that act as immediate stimuli. Together, these mediators raise the total amount of white blood cells and help to create more of them, multiply, mature, and release heterophils.

Nearly, the same results were obtained by (18,7) which indicates that heterophils are the first line of defense that led to leukocytosis and heterophilic which are

responsible for phagocytosis of infected microorganism and damaged cells.

The results of biochemical analysis refer to an increase values of liver enzymes (AST and ALT). Same results were obtained by (18,19), who reported the inflammation or injury due to hepatic damage and release of enzyme from damaged cells into serum. Increased values of cardiac protein Troponin (Tn), these ideas may agree with (2,20), this explains the infection by *E. coli* may be because of damage to the heart muscle cells' release of cardiac protein into serum; as well as inflammation of the pericardial cavity of the heart due to fibrinous pericarditis infected microorganisms and damaged cells.

The results of macroscopically shows severe accumulation of white typical fibrinous exudate in the pericardial sac referring to severe fibrinous pericarditis, also there is an enlargement of the congested liver (hepatomegaly) with obvious white typical fibrinous exudate in the parietal surface of the liver referring to fibrinous perihepatitis, also observed by (21).

In the present study, the outcome of histopathological changes of the infected heart showed severe infiltration of inflammatory cells in the pericardial sac with fibrinous exudation, also there is an edematous exudate in the myocardial muscle fibers, another section of the heart showed severe infiltration of polymorphonuclear inflammatory cells in the area between the pericardium and the myocardial muscle fibers. In addition, there are necrotic foci of myocardial muscle fibers. A

histopathological change of the liver of infected chicken showed thick severe fibrinous exudate capsulated on the liver surface multiple micro-abscesses in the hepatic parenchyma and dilation of sinusoids. The acute inflammatory reaction caused by beta hemolysin toxin secreted by *E. coli* led to increase in vascular permeability and this strong injury lead to fibrinous exudate (fibrinogen) escaping to the surrounding tissue, which is one of the most important causes of fibrinous pericarditis and perihepatitis in chickens. This lesion is in agreement with the findings of (22,23) who described the inflammation of the pericardium and liver capsule produces a serous or purulent discharge, the inflammatory exudate and influx of heterophils resulting in a fibrinous reaction with adhesion and fluid accumulation.

The histochemical section of the heart of the infected chicken of the present study using Mallory trichrome stain showed collagen and fibrin fibers that extensively accumulate in the pericardial sac referring to active fibrinous pericarditis. While the myocardial muscle fibers appeared yellowish. Nearly similar result clarified by (24), who used the Mallory stain to estimate the colors of collagen fiber. Histochemical section of the infected liver using Mallory's trichrome stain which showed staining of collagen and fibrin fibers that extensively accumulate in the hepatic capsular region referring to active fibrinous perihepatitis. Our observations are in agreement with (25) who reported the special stain (Mallory's trichrome stain) for showing collagen-rich connective tissue and the guess of the degree of fibrosis in hepatic capsule, Immature

fibrous tissue is pale blue, while mature fibrous tissue is dark blue.

### Conclusions:

According to the results of the current study, heterophils and lymphocyte numbers significantly increased in fibrinous pericarditis and fibrinous perihepatitis compared with controls, as well as in total differential white blood cell count. Additionally, compared with the control, the biochemical study showed a significantly higher concentration of ALT, AST, and troponin titer. Histopathological examination revealed infiltration of inflammatory cell and fibrinous exudate in both pericardial sac and hepatic capsular. Histochemical abnormalities, thick fibrin fibers that appeared positively in Mallory trichrome stain.

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## بعض الدراسات الفسيولوجية والمرضية لالتهاب التامور الفبريني والتهاب حوائط الكبد عند تعرض الدجاج لالتهاب الحويصلات الهوائية

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### الخلاصة

أجريت الدراسة الحالية للكشف عن التهاب التامور الليفى والتهاب حوائط الكبد في دجاج التسمين والبياض المصابة طبيعياً في محافظة البصرة، العراق ، النتائج السريرية المرضية ، وتغيرات الدم. تم الحصول على العينات المشتبه بها من عدة مواقع في محافظة البصرة (الهارة و ابي الخصيب والقرنة). أظهرت نتائج هذه الدراسة أن بعض الطيور ظهرت عليها أعراض سريرية مثل انخفاض التغذية في الدجاج، وفقدان الوزن، وتكدس الريش، بالإضافة إلى صعوبة التنفس، والسعال، وضعف الوعي. كشف فحص الدم عن زيادة معنوية في خلايا الدم البيضاء والخلايا غير المتجانسة والخلايا الليمفاوية. اظهرت نتائج مصل الدم عن زياده معنوية في AST و ALT والتروبونين) مقارنة بالطيور السليمة. ( $P \leq 0.05$ ) أظهر الفحص العياني للأعضاء الداخلية تراكمًا شديدًا للإفرازات الليفية النموذجية البيضاء في كيس التامور، كما يوجد تضخم للكبد مع إفراز ليفي نموذجي أبيض واضح في السطح الجداري للكبد. يُظهر المقطع النسيجي المرضي للقلب ارتشاحًا شديدًا للخلايا الالتهابية في كيس التامور مع إفراز ليفي ، كما يوجد إفرازات متوذمة في ألياف عضلة القلب. يُظهر القسم النسيجي المرضي للكبد إفرازات ليفية شديدة كثيفة مغلقة على سطح الكبد. يُظهر القسم الكيميائي للنسيج في القلب والكبد تليخًا إيجابيًا مزرقًا لألياف الكولاجين والفيبرين التي تتراكم في كيس التامور وفي المحفظة الكبدية. انتهت الدراسة إلى أن التهاب التامور الفبريني والتهاب حوائط الكبد يؤديان إلى التأثير على الحالة الصحية والأداء لأنواع مختلفة من الدجاج كالفروج أو البياض.

**الكلمات المفتاحية:** امراض الدم، الكيمياء الحيوية، علم امراض الانسجة، الدجاج.