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GENOTYPIC CHARACTERIZATION OF *Escherichia coli* ISOLATED FROM INFECTED CHICKEN IN BASRAH, IRAQ

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ABSTRACT: This study aimed to detect the presence of *Escherichia coli* in broiler and layer hens in the Basrah province, Iraq using macroscopic and microscopic diagnosis and bacterial isolation that causes infection in some internal organs (liver and heart), and by polymerase chain reaction. Randomly chosen samples were taken from different places within Basrah province for further investigation (poultry fields in Al-Qurnah and Al-Hartha). The bacteriological analysis revealed that the presence of *Escherichia coli* is responsible for causing fibrinous pericarditis and perihepatitis in birds. The macroscopic examination revealed hemorrhagic lesions and a significant buildup consisting of a white fibrous accumulation in the pericardial sac of the infected birds' hearts. The livers of infected birds exhibited significant deposition of white fibrous exudate on the liver surface, along with hepatomegaly. The afflicted heart displays a microscopic appearance marked by a notable aggregation of inflammatory cells in the pericardial sac and the release of fibrinous exudate. Additionally, there is an accumulation of edematous exudate in the cardiac muscle fibers, accompanied with congestion of blood vessels in the myocardium. The microscopic examination of the infected liver revealed the existence of a significant infiltration of inflammatory cells in the liver capsule, as well as the presence of a thick fibrinous exudate encapsulated on the liver surface and congestion of the central vein. The histological analysis of the affected heart and liver revealed a significant buildup of collagen and fibrin fibers, which exhibit a prominent dark bluish staining. This buildup is widely distinguished in the pericardial and hepatic capsules. The study indicated that fibrinous pericarditis and perihepatitis affected birds, as indicated by the examination of bacterial results. *Escherichia coli* emits endotoxins that induce vascular damage in the heart and liver, resulting in an elevated presence of fibrin exudate around the affected tissue. The histological analysis supported this conclusion.

Keywords: Fibrinous, Pericarditis, Perihepatitis, Pathology, Biological.

INTRODUCTION

Fibrinous pericarditis is inflammation of the pericardium that is accompanied by hyperemia and the deposit of fibrin within the pericardial sac (Perkins et al., 2004). Fibrinous perihepatitis is inflammation of the hepatic capsule by the accumulation of large amounts of There is a layer of fibrinous exudate covering the liver's surface. consisting of heterophils and lymphocytes (Bhalerao et al., 2013).

Fibrinous pericarditis and perihepatitis in poultry associated with colibacillosis and mycoplasmosis cause Issues pertaining to the economy and well-being of chickens. The frequent incidence of this phenomenon had detrimental impacts on both growth and health status. Termed airsacculitis or chronic respiratory disease in medical terminology, this condition leads to respiratory discomfort, stunted growth, reduced food consumption, and an increased mortality rate. *Escherichia coli* infections are often concurrent and result in exudative accumulations, adhesive fibrinous pericarditis, and fibrinous perihepatitis (Vandemaele et al., 2002).

Pneumonia and airsacculitis may eventually allow for vascular system entry. The primary source of systemic colibacillosis or colisepticemia is thought to be this aerogenic route of infection (Dho-Moulin and Fairbrother, 1999). Colisepticemia is characterized by *E. coli* in the circulation (Pourbakhsh et al., 1997). Airsacculitis, a respiratory infection that first develops in colisepticemia, is followed by a widespread infection that includes perihepatitis and pericarditis (Mellata et al., 2003).

Avian pathogenic *Escherichia coli* causes a variety of systemic or localized infections, including colisepticemia (fibrinous exudates being present in several organs), respiratory infections, airsacculitis, swollen head syndrome, peritonitis, salpingitis, yolk sack infections in newly hatched chicks, and skin infections (Nolan et al. 2013). *Escherichia coli* infections frequently occur concurrently and produce exudative accumulations, sticky pericarditis, and fibrinous perihepatitis in addition to significant air sac thickening and turbidity (Nolan et al., 2013).

Physical and biological risk factors that significantly increase the likelihood of colibacillosis in chickens include housing conditions and co-infections with other bacteria. Most diseases spread through aerosols and colonize air sacs (Lamarche et al., 2005). Dust and ammonia work together to produce harmful consequences, and the inhalation of dust

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contaminated with feces can result in respiratory illnesses caused by *E. coli*. Soon after the air in chicken houses contains high levels of *E. coli*, outbreaks of colisepticemia start to happen (Barnes and Gross, 1997).

The current study aimed to establish a relevant definition of vulnerability to colibacillosis requires more understanding of the relative severity of lesions as well as microbiological analysis of the main causative agents of fibrinous pericarditis and fibrinous perihepatitis based on confirmative methods. The primary distinctive macroscopic abnormalities of fibrinous pericarditis and perihepatitis have been recognized, and the distinctive lesions of fibrinous pericarditis and perihepatitis were examined microscopically. Specific stains were used to perform a histochemical study of fibrin deposition.

MATERIALS AND METHODS

The study concentrated on postmortem lesions, histological changes, and PCR for laboratory diagnosis confirmation. The current study lasted from October 2022 to March 2023. Out of 150 domestic bird samples were selected from several locations throughout the province of Basra (poultry fields in Al-Qurnah and Al-Hartha) according on presence of clinical cases. The postmortem lesions were prepared in processed steps according to Davis and Morishita (2001). The current study was performed under the permission of the ethical committee in the Faculty of Veterinary Medicine, University of Basrah (Ref. No. 79/2023).

Culturing

Swabs were obtained by using sterile cotton swabs from fibrin material on the liver surface and heart surface in an attempt to isolate *E. coli* then placed in test tubes containing nutrient broth and incubated at 37°C for 24 hours, culture was streaked onto plates of MacConkey agar (MC), Eosin methylene blue agar (EMB) and blood agar. The dishes were then incubated aerobically for 24 to 48 hours at 37 degrees Celsius, Subsequently; the bacteria that were cultivated on the same medium were isolated and purified. and separated and preparation for the polymerase chain reaction (PCR) process (Ali and AL-Mayah, 2015).

Genetic Identification

Genomic DNA extraction

The genomic DNA purification kit (Promega/USA) was used to extract the DNA from the ten isolated *E. coli* bacteria. The outcome was identified using electrophoresis on a gel consisting of 1.5% agarose and revealed the DNA bands under ultraviolet light were visualized (Jaber, 2019).

Polymerase chain reaction (PCR)

Detection of the DNA of *E. coli* bacteria was performed by PCR with master mix and specific primers as in Table 1 according to Corp (2005).

Purification and sequencing

The PCR products of the 16S rRNA gene were sequenced by Macrogen Company (South Korea) for comprehensive identification isolates of *E. coli* bacteria.

Histopathological analysis

Tissue sample taken from heart and liver were fixed in 10% buffered formalin to fix for 72 hours and tissue were embedded in paraffin blocks and then routine tissue procedures divided into sections with a thickness of 5 microns and then stained with Haematoxylin & Eosin. The sections were examined under a light microscope according to Suvarna *et al*. (2018) and Ahmed (2020). Masson's trichrome stain was also used to detect fibrin in tissue sections (Khismatullin et al. 2020).

Table 1 - Universal 16S rRNA primer used in PCR amplification of *E. coli* bacreria

Gene	PCR Primers	Nucleotide sequence (5' → 3')	Base pairs
16S rRNA	Forward	AGAGTTTGATCMTGGCTCAG	1500
	Reverse	CCGTCAATTCCTTTRAGTTT	

RESULTS

Bacteriological results

Isolation of *E. coli* bacteria that cause fibrinous pericarditis and perihepatitis in birds. Based on the culturing of heart and liver swaps on MacConkey agar, eosin methylene blue agar, and the use of blood agar. The distribution of the *E. coli* bacterial isolation results is as follows: out of a total of 150 samples, from each heart and liver sample that were collected from the infected broiler 80/90 (88.88%), while the cultured and isolated samples from the infected layer 30/60 (50%) as in Table 2.

PCR-based molecular identification

Extraction and detection of DNA

Ten isolated *E. coli* The DNA of the bacteria was isolated, separated using electrophoresis with a 1.5% agarose gel, and seen using UV light. Subsequently, the 16S rRNA was amplified using the polymerase chain reaction (PCR) technique, resulting in the identification of a clearly distinguishable gene band of 1500 base pairs, as depicted in Figure 1.

Sequencing of 16s rRNA and *E. coli* bacterial identification

Ten *E. coli* were isolated from chicken liver and heart tissue during the bacteriological examination of fibrinous pericarditis and perihepatitis. The results of 16S ribosomal RNA nucleotide sequencing of all investigated *E. coli* strains isolates were registered in NCBI-BLAST under sequence ID as in Table 3.

Table 2 - The data provided pertains to the quantity and proportion of avian specimens that have tested positive for infection, namely in heart and liver samples obtained from both broiler and layer birds.

Birds	No. of birds	No. of infected birds	% of infected
Broiler	90	80	88.88%
Layer	60	30	50%

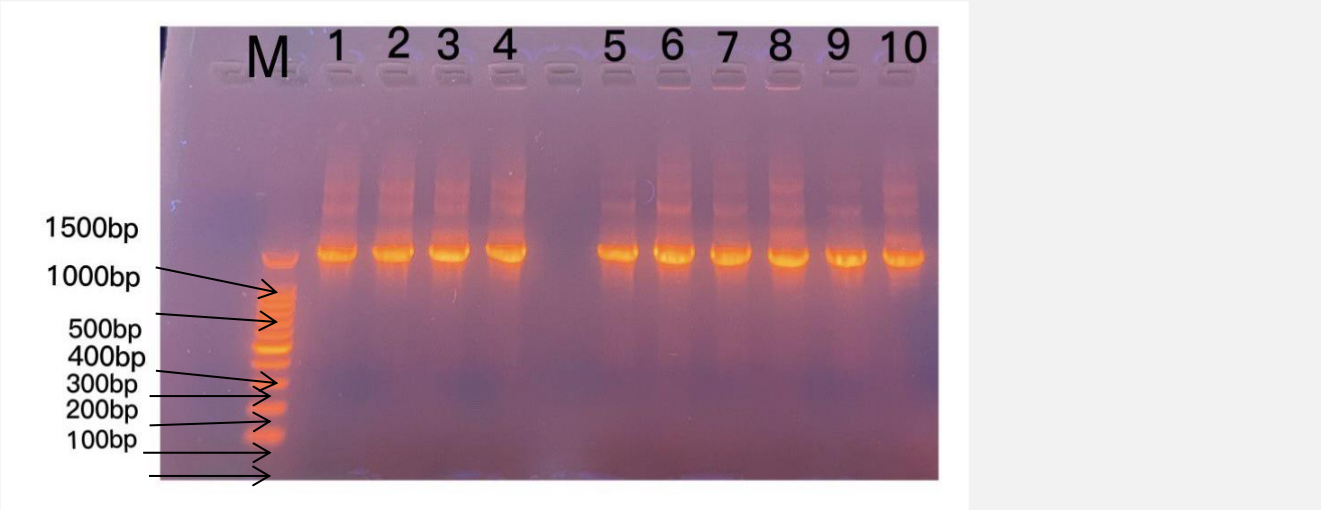

Figure 1 - Using agarose gel electrophoresis, the PCR product analysis of 16S rRNA amplification in the 1500 bp region may be seen.

Table 3 - Identified *Escherichia coli* strains by16S rRNA gene sequencing

Number	Source	Sequence ID with Submission	Sequence ID with compare	Identities
1	*Escherichia coli* Strain Bu.Ji.Ha.1.IRAQ	ID: OQ954793.1	ID: OP630887.1	99%
2	*Escherichia coli* Strain Bu.Ji.Ha.2.IRAQ	ID: OQ954794.1	ID: OP630887.1	99%
3	*Escherichia coli* Strain Bu.Ji.Ha.3.IRAQ	ID: OQ954795.1	ID: OP630887.1	99%
4	*Escherichia coli* Strain Bu.Ji.Ha.4.IRAQ	ID: OQ954796.1	ID: OP630887.1	99%
5	*Escherichia coli* Strain Bu.Ji.Ha.5.IRAQ	ID: OQ954797.1	ID: OP630887.1	99%
6	*Escherichia coli* Strain Bu.Ji.Ha.6.IRAQ	ID: OQ954798.1	ID: OP630887.1	100%
7	*Escherichia coli* O104:H4 Strain Bu.Ji.Ha.7.IRAQ	ID: OR082830.1	ID: CP031902.1	99%
8	*Escherichia coli* Strain Bu.Ji.Ha.8.IRAQ	ID: OR082831.1	ID: OQ891229.1	99%
9	*Escherichia coli* O157:H7 Strain Bu.Ji.Ha.9.IRAQ	ID: OR082832.1	ID: CP039834.1	99%
10	*Escherichia coli* Strain Bu.Ji.Ha.10.IRAQ	ID: OR082833.1	ID: OQ753150.1	99%

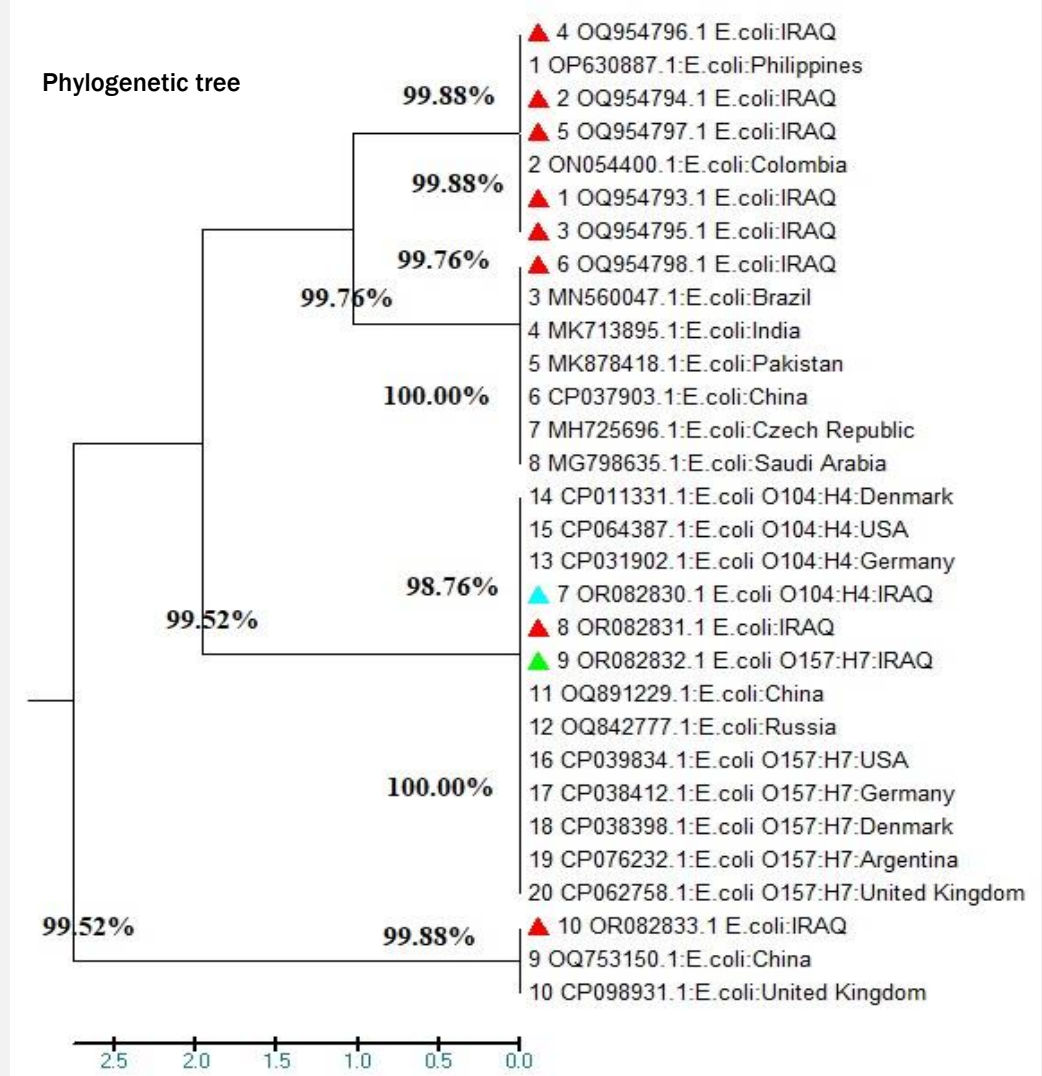

Figure 2 - A maximum likelihood tree illustrates the evolutionary relationship between Escherichia coli's 16S rRNA sequence and 16S rRNA sequences of closely related bacterial species. *Escherichia coli* were isolated from a local chicken heart and liver in Iraq. Their accession numbers are used to express them in international nucleotide databases. The phylogenetic tree was produced using Mega 6 sequencing, version 6.5 software.

Pathological manifestations

Macroscopic findings

The pericardial sac of the sick chicken showed a notable buildup of white fibrinous exudate within its internal organs, indicating a serious case of fibrinous pericarditis. Additionally, the liver showed enlargement and congestion (hepatomegaly), with visible white fibrinous exudate on the outer surface, indicating fibrinous perihepatitis as shown in Figure 3. The hearts of hens affected by the illness exhibited substantial accumulation of white fibrinous debris in the sac surrounding the heart, indicating the presence of severe fibrinous pericarditis. Additionally, there was a hemorrhagic lesion on the surface of the pericardium, indicative of hemorrhagic pericarditis as depicted in Figure 4. The liver of the diseased chicken had a pronounced buildup of white fibrinous exudate on its surface, indicating severe fibrinous perihepatitis. Additionally, the liver was enlarged, known as hepatomegaly, as depicted in Figure 5.

Microscopic results

The histopathological examination of the chicken's infected heart shows fibrinous exudate in the pericardial sac and a significant infiltration of inflammatory cells with fibrinous exudation in the pericardium. Furthermore, there is a necrotic region in the myocardial muscle fibers (Figure 6). In addition to demonstrating the presence of many active micro-abscesses in the pericardium, the pericardium also exhibits a notable infiltration of inflammatory cells with fibrinous exudation, as well as congestion of blood vessels in the myocardium (Figure 7). The histological examination of the chicken's liver reveals a significant presence of inflammatory cells in the hepatic capsular region. Additionally, there is a thick layer of fibrinous exudate encapsulated on the surface of the liver, as depicted in Figure 8. In addition to the central vein being congested with active inflammatory exudate (shown by the black arrow), there is also extensive perivascular necrosis of the hepatocytes (Figure 9).

Histochemical findings

The examination of the heart tissue from the infected chicken revealed a notable accumulation of pericardial collagen and fibrin, indicating the presence of active fibrinous pericarditis. These fibers stained dark bluish in color. Additionally, the myocardial muscle fibers appeared pinky-red when stained with Masson's trichrome (Figure 10). The liver section of the infected chicken exhibits a significant buildup of collagen and fibrin fibers in the capsular hepatic region, indicating active fibrinous perihepatitis. This is evident from the light bluish positive staining observed in the histochemical analysis. Additionally, the hepatic parenchyma appears pinky-red in color when stained with Masson's trichrome, (Figure 11).

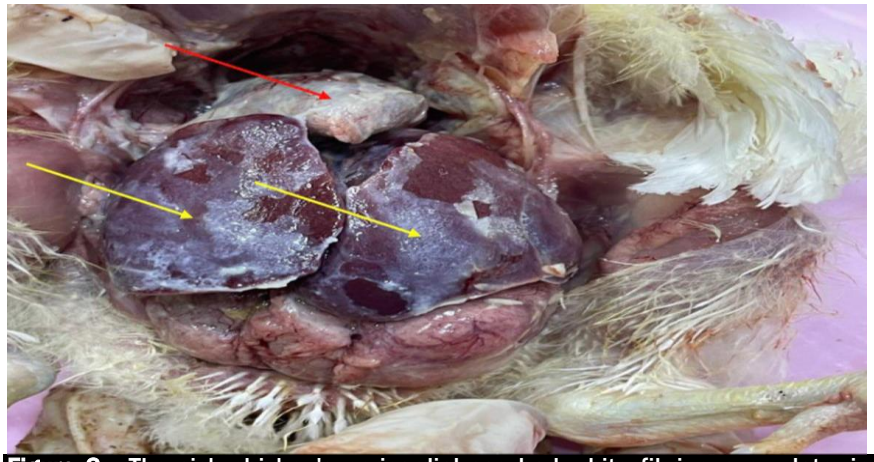

Figure 3 - The sick chicken's pericardial sac had white fibrinous exudate, indicating severe fibrinous pericarditis (red arrow). The enlarged liver (hepatomegaly) has white fibrinous exudate on its outer membrane, indicating fibrinous perihepatitis (yellow arrow).

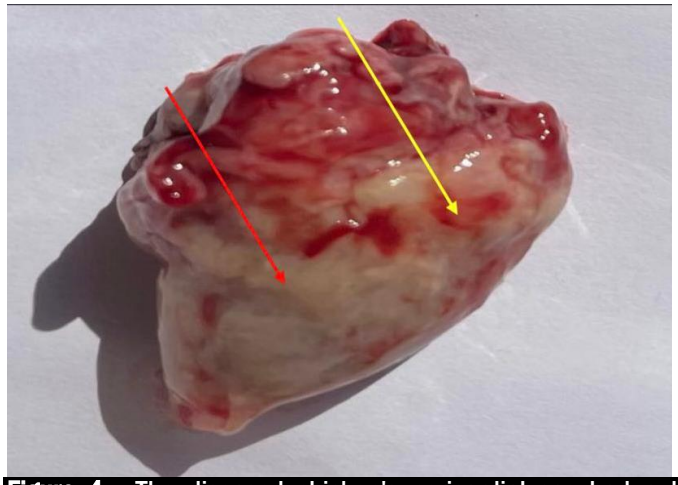

Figure 4 - The diseased chicken's pericardial sac had a lot of white fibrinous exudate, indicating severe fibrinous pericarditis (red arrow). The liver was enlarged (hepatomegaly) and had white fibrinous exudate on the outside, indicating fibrinous perihepatitis (yellow arrow).

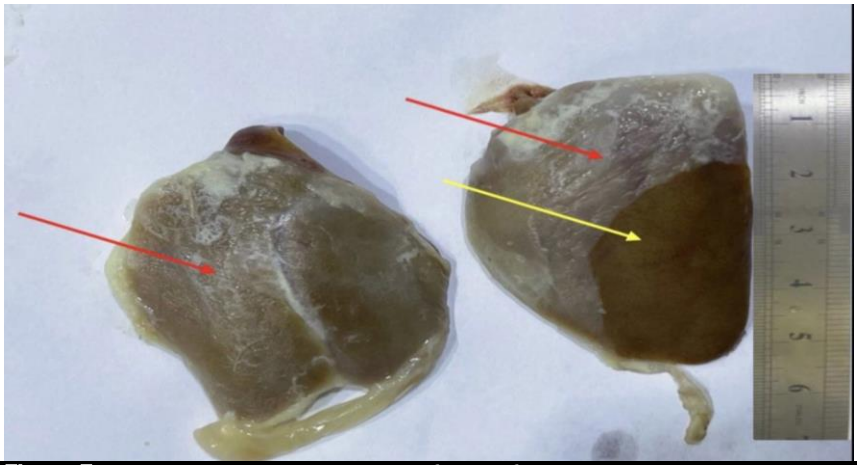

Figure 5 - The chicken's liver had a lot of white fibrinous exudate, indicating severe fibrinous perihepatitis (red arrow). Liver enlargement indicated hepatomegaly (yellow arrow).

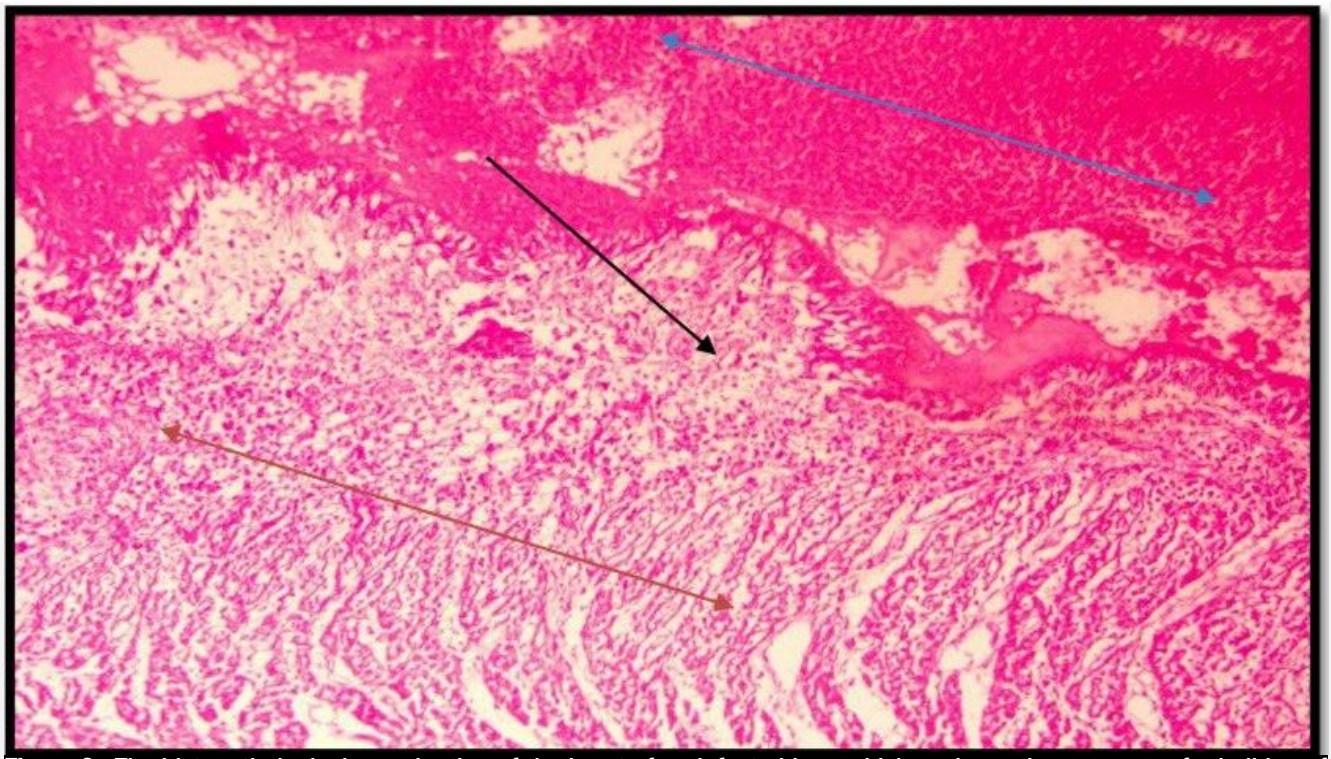

Figure 6 - The histopathological examination of the heart of an infected layer chicken shows the presence of a buildup of fibrinous exudate in the pericardial sac (shown by a double-headed blue arrow), along with a significant infiltration of inflammatory cells in the pericardium and the presence of fibrinous exudation. The presence of a black arrow indicates the existence of a necrotic region within the cardiac muscle fibers, as indicated by the double-headed red arrows. Hematoxylin and eosin stain. One hundred times.

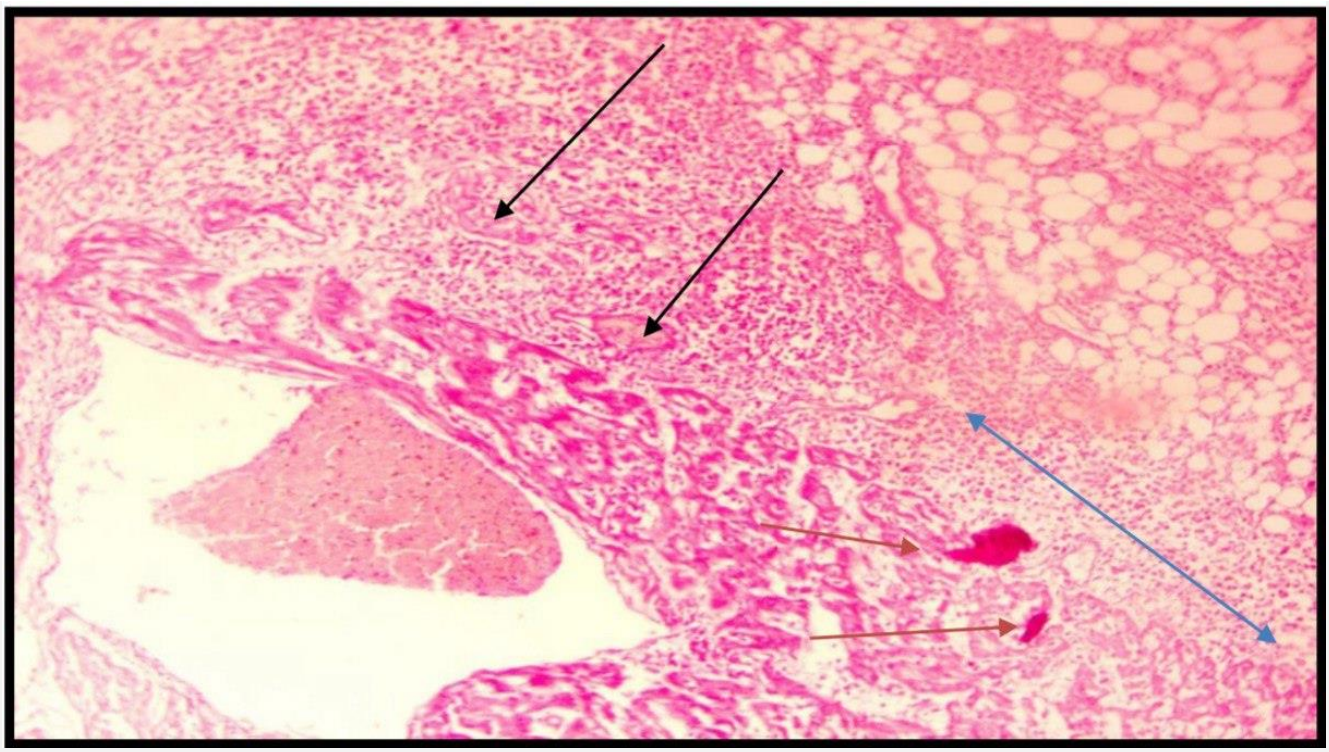

Figure 7 - The histopathological image of the chicken's heart reveals the presence of numerous active micro-abscesses in the pericardium (black arrows). Furthermore, the pericardium displays infiltration of inflammatory cells and the release of fibrinous exudate (indicated by a double-headed blue arrow), along with blood vessel congestion in the myocardium (red arrows). Hematoxylin and eosin stain at a magnification of 100X.

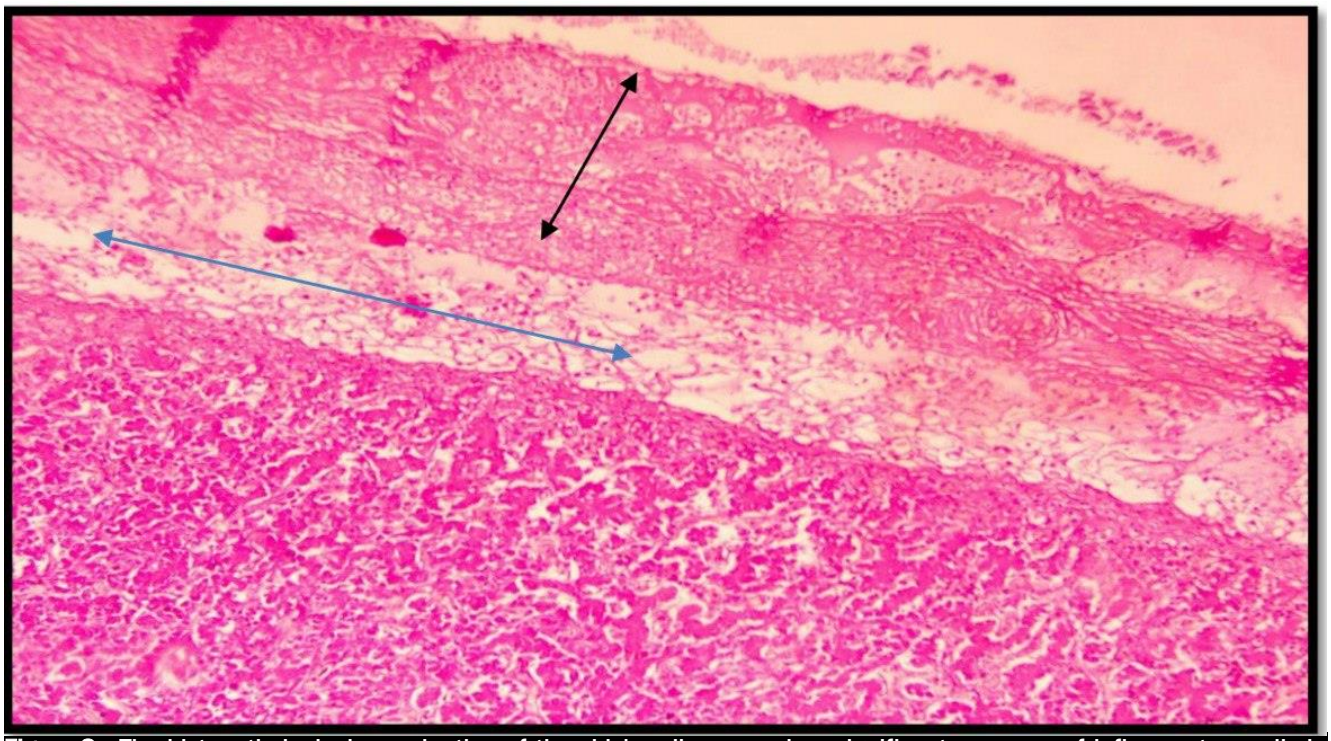

Figure 8 - The histopathological examination of the chicken liver reveals a significant presence of inflammatory cells in the outer layer of the liver (double-headed blue arrow). Additionally, there is a thick layer of fibrinous exudate covering the surface of the liver (double-headed black arrows). Hematoxylin and eosin stain. Magnification of 100 X.

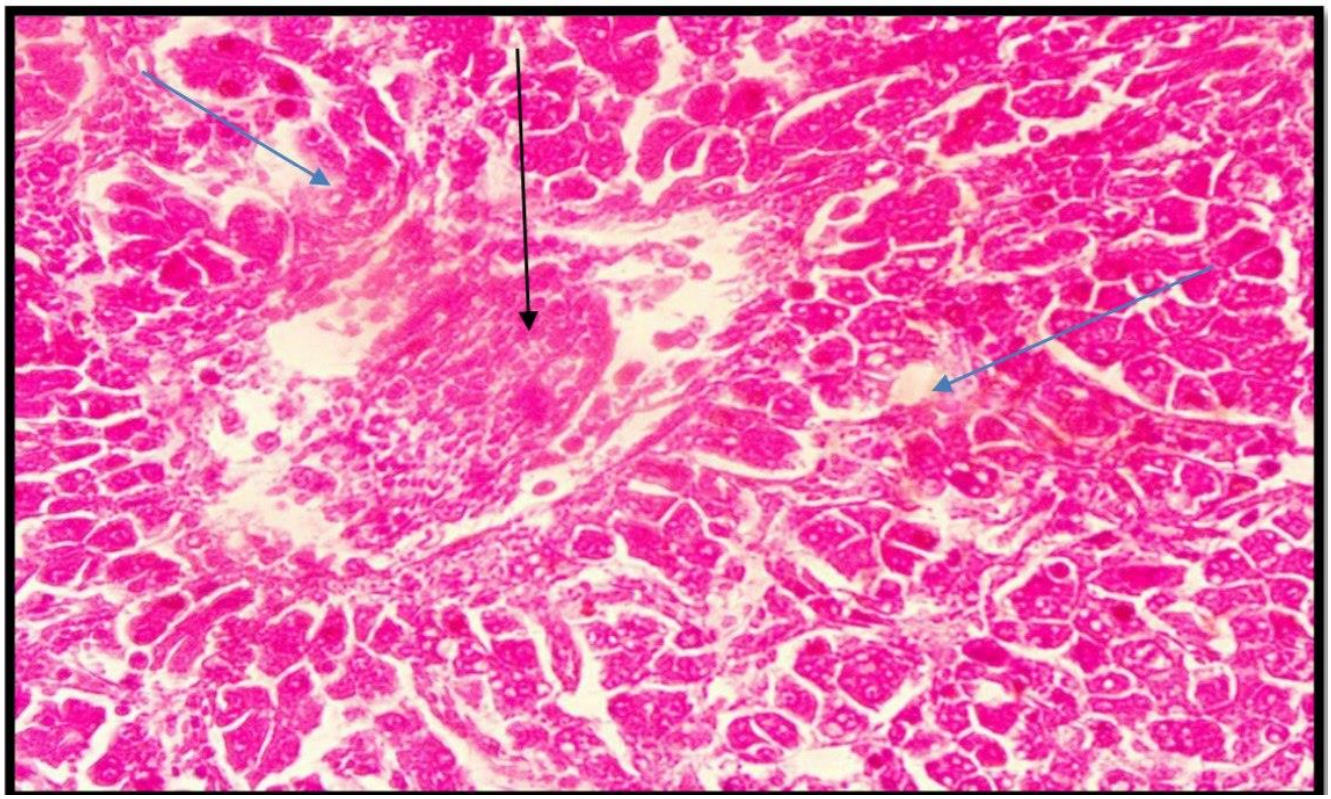

Figure 9 - Microscopic image depicting the liver tissue of a chicken that has been infected. reveals congestion of the central vein with active inflammatory exudate (black arrow), also there is severe perivascular necrosis of the hepatocytes (blue arrows). H&E stain. 40X

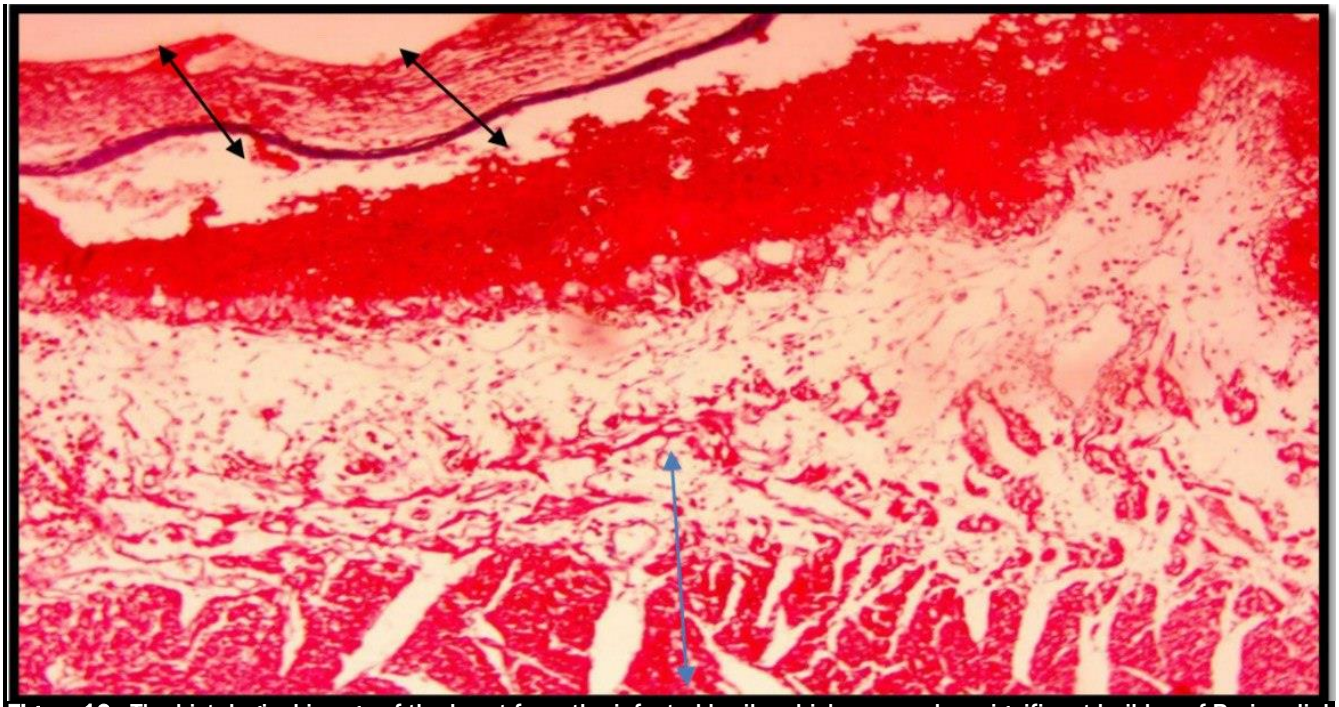

Figure 10 - The histological image of the heart from the infected broiler chicken reveals a significant buildup of Pericardial collagen and fibrin, indicating the presence of active fibrinous pericarditis. These fibers are stained dark bluish. In contrast, the myocardial muscle fibers are observed in a pinky-red color. Masson's trichrome stain is a histological staining technique 100X.

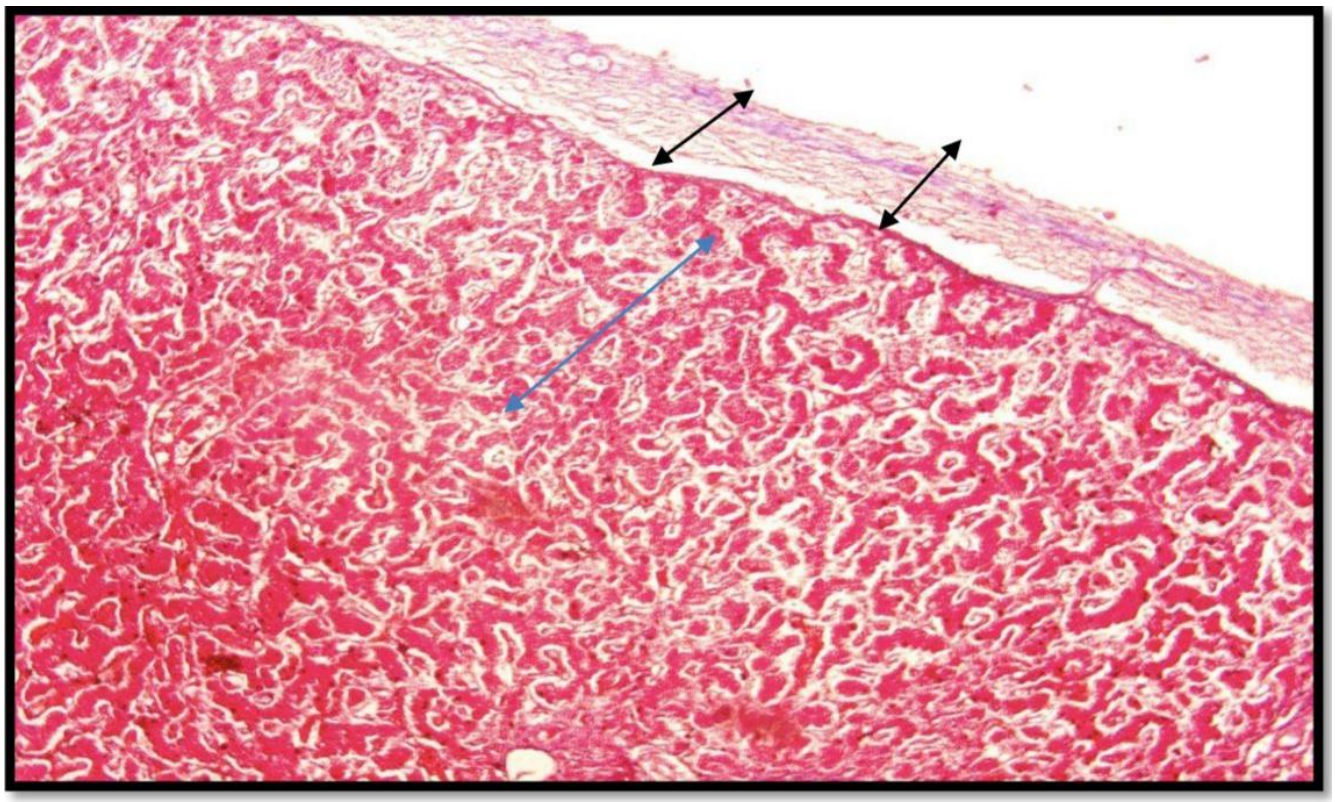

Figure 11 - The ill broiler chicken's hepatocyte cells revealed light bluish positive staining of collagen and fibrin fibers that build up in the capsular hepatic region, indicating active fibrinous perihepatitis. The hepatic parenchyma was pinky-red (double-headed blue arrow). Masson trichrome 100X

DISCUSSION

The results of bacterial culture on MacConkey, EMB, and blood agar revealed that the heart and liver samples contracted an infection with *E. coli,* our results are in agreement with research by Wani et al. (2020) which discovered that *E. coli* colonies showed a metallic sheen on EMB agar and appeared pink when cultured on MacConkey agar plates, Due to its ability to ferment lactose and the formation of an amide linkage between eosin and methylene, *E. coli* colonies are pink in color and show a metallic sheen on EMB. Their findings revealed the presence of *E. coli* bacteria, which cause pericarditis and perihepatitis.

According to the results, the broiler had a larger percentage of E. coli bacterial isolation than the layer, with infection rates of 88.88% and 50%, respectively. The current study's findings concurred with those of Mohanty et al. (1979) and Ezz El-Deen et al. (2010), who found the *E. coli* bacteria in infected broilers and layers, respectively, with an incidence of 88.8% and 75%. Furthermore, these results align with the research conducted by Dho-Moulin and Fairbrother (1999), which found that fibrinous pericarditis and fibrinous perihepatitis are caused by *E. coli* epithelium penetrating the mucosa of the respiratory organs and multiplying in the bloodstream and internal organs (liver and heart).

As indicated in Table 3 and Figure 2, which depicts the distribution of Iraqi samples, the current work employed neighbor-joining analysis of the 16S rRNA gene to generate a phylogenetic tree in order to investigate the relationship between local samples and the higher query cover (99%) of national samples, in this Figure 2 indicates the phylogenetic tree for *E. coli* (No. 1- 6) display related similarity (99.88%) with the sample from the Philippines, while *E. coli* (No.7) display related similarity (99.52%) with the sample from Germany; while *E.coli* (No.8) indicates maximum similarity (100%) with the sample from China. Also, *E. coli* (No.9) indicates maximum similarity (100%) with the sample from the USA, while *E. coli* (No.10) display related similarity (99.88%) with the sample from China, according to (Zhang and Sun, 2008). The majority of isolates of the genetically heterogeneous species *Escherichia coli* are commensal digestive system organisms. However, some isolates are opportunistic pathogens that infect a range of hosts' extra intestinal and gastrointestinal systems (Denamur et al., 2021).

The macroscopic analysis revealed that diseased birds with fibrinous pericarditis and perihepatitis exhibited a significant buildup of white, characteristic fibrinous exudate in the pericardial sac, together with hemorrhagic lesions on the surface of the pericardium. *E. coli* infection of the heart causes damage to the heart blood vessels resulting in hemorrhage. This result agrees with Pruthi et al. (2012); Bhalerao et al. (2013) who mentioned that the fibrinous layer on the pericardium and hemorrhagic due to adhesions of the heart with the chest cavity.

Also, severe accumulation of white typical fibrinous exudate in the liver surface and enlargement of the liver. This in line with Dutta et al. (2013) who found that the deposition of large amounts of fibrinous exudate on the liver, a bacterial infection of the liver causes inflammation with a large number of heterophils over the hepatic capsule due to enlargement of the liver (hepatomegaly).

The microscopical observation in the heart changes appeared including fibrinous exudation and congestion of blood arteries in the myocardium, pericardial sac infiltration of inflammatory cells, edematous exudate in the cardiac muscle fibers, and numerous active micro-abscesses in the pericardium. Regarding the further alterations, they include a significant polymorphonuclear inflammatory cell infiltration in the region that lies between the cardiac muscle fibers and the pericardium. Damage to the pericardium brought on by bacterial infections results in the release of fibrin, an inflammatory cell. This lesion is consistent with the observations made by Snyder et al. (2014), who reported that pericardial inflammation results in a serous or purulent discharge, inflammatory exudate, and heterophil inflow, which causes a fibrinous reaction with adhesions and fluid accumulation.

The contaminated chicken's liver exhibits severe inflammatory cell infiltration in the capsular hepatic region, as well as thick fibrinous exudate capsulated on the liver surface, inflammatory cell infiltration of the liver parenchyma, abscesses in the hepatic parenchyma, and dilation of the sinusoids. Also, there are foci of necrosis in the hepatocytes. *E. coli* release beta hemolysin toxin that causes increased vascular permeability and escape of inflammatory cell and fibrinogen due to accumulation of fibrinous exudate in the surrounding tissue, and due to lack of blood supply to tissues and cell death (necrosis). This result was in lined with Dutta et al. (2013), Kadhim and Ahmed (2020) and IRufaei and Alwan (2023) who noted that the liver showed localized necrosis in the hepatocytes as well as a thick layer of fibrinous exudates covering the hepatic capsule and a large number of heterophils.

The heart histochemical section of the infected chicken in this study, stained with Masson's trichrome stain, revealed a significant accumulation of collagen and fibrin fibers in the pericardial sac, indicating active fibrinous pericarditis. The myocardial muscle fibers showed up pinky-red in color. These findings were similar to that of Franca et al. (2010) who described the accumulation of collagen fibers in the heart (pericardium and myocardium) that appear in different colors when stained with Masson's trichrome stain.

The histological analysis of the liver using Masson's trichrome stain revealed a significant accumulation of collagen and fibrin fibers in the capsular hepatic region, indicating the presence of active fibrinous perihepatitis. These fibers were stained pale bluish. In contrast, the hepatic parenchyma appeared pinky-red in color. This observation was also documented by Krishna (2013), who discovered that the stain imparts a blue hue to collagen in contrast to the red color of hepatocytes and other structures.

CONCLUSION

Overall, our study revealed fibrinous pericarditis and perihepatitis were higher in broilers than in layers, as well as the bacteriological study showed the of *Escherichia coli* which cause fibrinous pericarditis and perihepatitis, as well as white fibrinous exudate accumulated in the pericardial sac and on the liver's surface, as well as microscopic examination of fibrinous pericarditis revealed infiltration of inflammatory cells with fibrinous exudation, edematous exudate congestion of blood vessels in the myocardial muscle fibers with the presence of a necrotic area, and multiple active micro-abscesses in the pericardi, as well as microscopical examination of fibrinous perihepatitis revealed infiltration of inflammatory cells and thick fibrinous exudate in the liver capsule, as well as congestion of the central vein with active inflammatory exudate also there is severe perivascular necrosis of the hepatocytes, as well as the histochemical section of fibrinous pericarditis and perihepatitis revealed the proliferation of collagen and fibrin fibers in the pericardial sac and capsular hepatic region.

DECLARATIONS

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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution
B.M. Lateif performed genetic analysis of the results and the manuscript's writing.
J.A. Ahmed performed molecular and pathological detection.
H.A. Najem contributed to the design of the research and field diagnosis of the disease.

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Consent to publish
The authors agree to the publication of this manuscript.

Competing interests
The authors declare no competing interests.

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