Physiological and histopathological study of chronic respiratory disease infected chickens in basrah city

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Abstract

Chronic respiratory disease is one of the economically important diseases that affects poultry and causes high mortality, and this negatively affects the poultry industry. Mycoplasma is the main cause of chronic respiratory infection in addition to presence of E.Coli in such disease , and coli bacteria play a role in the emergence of some complications, the current research aimed to study the physiological and histological effects in Broiler chickens with chronic respiratory disease,the samples were collected from the infected broiler chick farms and from veterinary clinics and diagnosed clinically, then blood was drawn and some tissues were fixed to prepare it for histopathological . The results showed a significant decrease in the number of Red blood cells(RBC), the rate of Hemoglobin(Hb), the hematocrit(Hct), and a significant increase in the number of white blood cells(WBC), liver enzymes(AST),(ALT), and Cardiac protein (Tn) for infected chickens compared to the control group. Histopathological sections were exhibited congestion, inflammation, vacuolation, and necrosis in the liver and heart in chickens infected with chronic respiratory disease, compared to that of control.

Keywords: *CRD, physiological parameters, histopathological, chickens.*

INTRODUCTION

Poultry farming, such as chickens and ducks, is an investment resource, as it provide eggs and meat, in addition to providing job opportunities for specialists and the unemployed, which constitute a large segmen of society, The demand for the poultry industry also increases greatly to provide high-quality proteins (Blake etal., 2014). But What hinders the development of this industry is the spread of diseases, which causes the death of about 30% of these birds every year (Ali, 1994). Among the most important of these diseases is chronic respiratory disease (CRD), and it is considered one of the diseases that cause great losses that lead to a decrease in production, whether meat

or eggs. Cold weather, poor ventilation or overcrowding, and lack of vaccination against viruses;those considered predisposing factors to infection. Clinical signs of chronic respiratory disease (CRD) are coughing, sneezing, difficulty breathing, foamy eye secretions, nasal secretions, conjunctivitis, loss of appetite, weight loss and eggs, weak embryos, mortality and difficulty hatching. (Charlton etal., 1996).Among the pathogens of chronic respiratory disease is Mycoplasma gallisepctum, which causes many diseases in poultry leading to large economic losses in poultry fields, and CRD is one of the most economically important diseases in the poultry industry, Mycoplasma gallisepticum is one of the organisms that are often colonized inside the respiratory tract and multiplies in the trachea, lung and alveoli. (Uddin etal., 2010). also show that the incidence of infection increases in summer, winter, and the rainy season (Yunus etal., 2009), while both Sultana etal. (2012) and Bahatti etal. (2013) mentioned that cases increase in winter, resulting in economic losses that include a large number of Mortality, low egg production, low hatching rates, weight, and undernourishment (Gondal etal., 2015 and Karthik etal., 2018). In addition to Mycoplasma gallisepticum, E. coli bacteria participate in the occurrence of complications of infection and damage. (Clark, 2019)and(Wakenell ,2016).

MATERIALS AND METHODS

The studied Samples were taken from broiler chicks farms which located in Al-Basrah province /Iraq ; the chicks with clinical signs and postmortem lesions related to chronic respiratory disease like ; rales,coughing ,conjunctivitis ,and lesions fibrinous pericarditis and pericarditis (figure 1,2) , Then blood was drown from wing vein from diseased and healthy chicks (figure 3,4), and then some organs such as the liver and heart were extracted(figure 5,6), They were placed in a 10% formalin solution,and embedded in paraffin wax andcut in to slices of 4- 5mm then stained with hematoxylin-eosin.(Allen,1994) and the histological effects of the disease were observed. The blood samples were divided into two parts, a group placed in tubes containing an anticoagulant for physiological blood tests, and the second group placed in tubes free of anticoagulants for biochemical tests.. Physiological tests included ,measure blood parameters, including (red blood cell count, white blood cell count, hemoglobin rate, and hematocrit.(Khan etal.,2013) ;(Chabot-Richards and George.,2015). As for the biochemical tests, three types of biomarkers were measured, Alanine aminotransferase (ALT), Aspartat-aminotransferase(AST), (Young ,2000), and cardiac protein Troponin(Tn). (Apple and Collinson.,2012) .

Figure (1) chicks with chronic respiratory disease

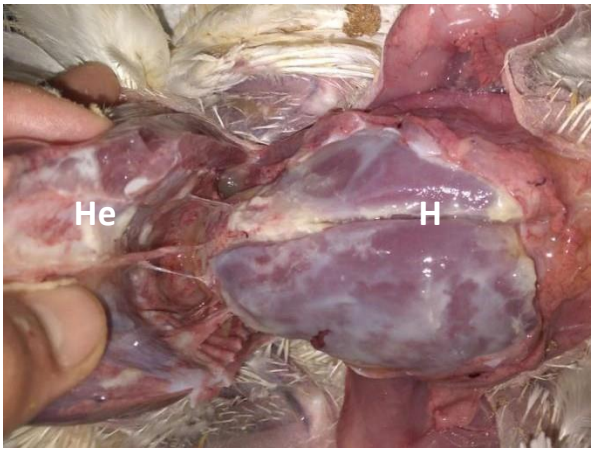
Figure (2) sluggishness and lethargy in infected chicks

Figure (3) shows the method of drawing blood from the extremities (wing).

Figure (4) shows the method of drawing blood from the extremities.

Figure (5) shows the liver of chicks infected with chronic respiratory disease (arrow).

Figure (6) shows the liver(H) and heart (He)of chicks infected with chronic respiratory disease



Results

1- Effect of chronic respiratory disease (CRD) on hematological parameters.

The results of the current study showed that there was a significant decrease at the level of probability ($P \leqslant 0.001$) in the number of red blood cells (RBC), hemoglobin rate (Hb) and Hematocrit (Hct). While the number of white blood cells (WBC) increased in the infected chicks compared to the control group. As shown In table (1).

Table (1) The effect of chronic respiratory disease (CRD) on hematological parameters

Hct %	Hb (Mg/dl)	WBC (10^3/mm^3)	RBC (10^6/mm^3)	Parameters / Treatment
42.035 .074±	13.96 0.023±	17.8 0.08±	3.61 .008±	Control
35.745* 0.11±	10.71* 0.046±	24.822* 0.073±	2.82* 0.016±	CRD

* There is a significant difference at the probability level(P≤ 0.001)

2- The effect of chronic respiratory disease on liver enzymes (ALT, AST) and cardiac protein (Tropnin).

The results showed that there was a significant increase at the level of probability (P≤0.001) in the liver enzymes, including Alanine aminotransferase (ALT), Aspartat-aminotransferase(AST), and cardiac protein Troponin(Tn), of the infective chicks, compared to the control group, as shown in Table (2).

Table (2) Effect of chronic respiratory disease on liver enzymes(AST,ALT) and Cardiac protein (Tn)

Tn Ng/ml	AST U/l	ALT U/l	parameters / Treatment
0.174 0.00086±	20.35 0.064±	22.43 0.071±	Control
0.34* 0.001±	40.71* 0.097±	32.34* 0.098±	CRD

* There is a significant difference at the probability level P≤ (0.001).

3- The effect of chronic respiratory disease in the tissues of the liver, heart .

Histological sections showed effects on the liver and heart of infected chicks, including:-

Figure (7) tissue section of chicks liver without chronic respiratory disease, showing hepatocytes(HC), Kupffer cells(KC), and venous sinusoids(S).(H&E).(400X).

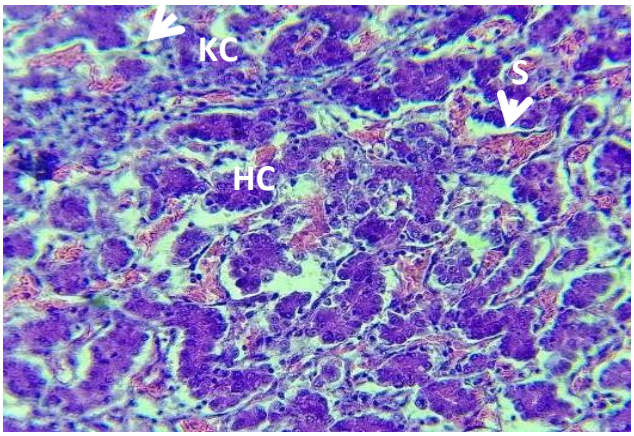


Figure (8) shows a histological section of chicks liver without chronic respiratory disease, showing the hepatic portal vein(HPV), hepatic artery(HA), and bile duct(B).(H&E).(100X).

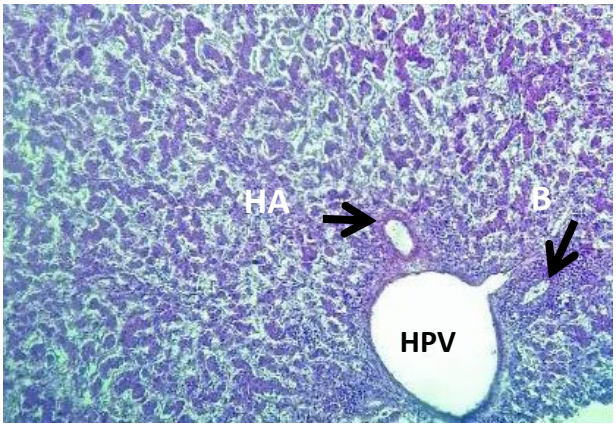


Figure (9) the liver of chicks without chronic respiratory disease, showing the central vein(CV)..(H&E).(400X).

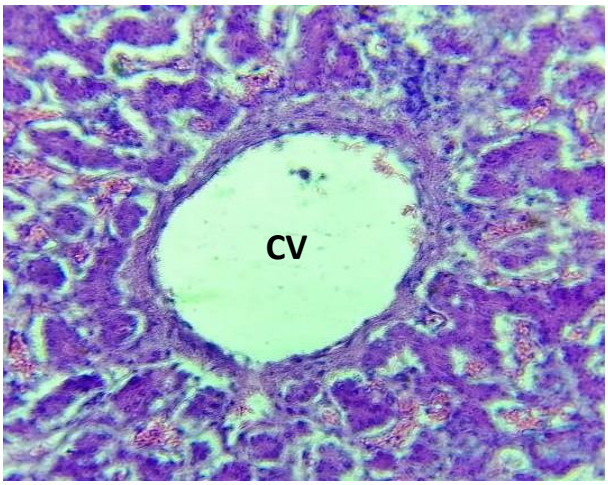


Figure (10) shows a tissue section of chicks liver that infected with chronic respiratory disease, noting inflammatory cells accumulate(INF) in the liver tissue.(H&E).(400X).

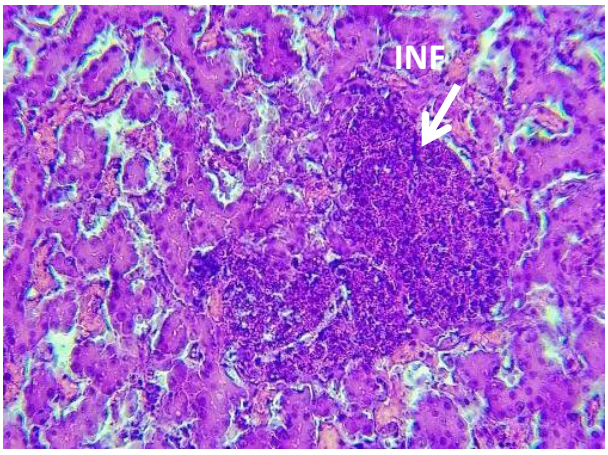


Figure (11) shows a histological section of the liver for chicks infected with chronic respiratory disease, noting the congestion of the central vein(CV) ..(H&E).(400X).

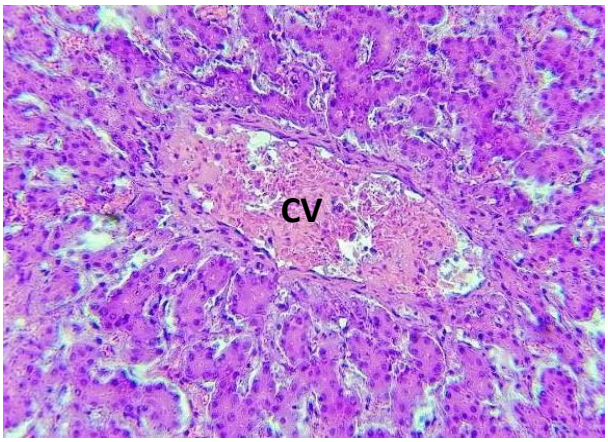


Figure (12) shows a tissue section of chicks liver with chronic respiratory disease, noting congestion of the hepatic central vein(CV) , inflammatory cells(INF) and necrosis gathering around it.(arrow) ..(H&E).(400X).

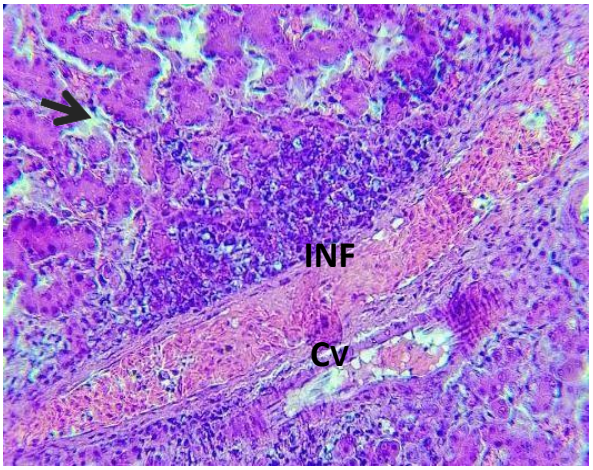


Figure (`13) shows a histological section of chicks liver with chronic respiratory disease, noting cell congestion(CON), vacuolation(V), and necrosis(N) in the tissue, with inflammatory cells gathering around it. ..(H&E).(400X).

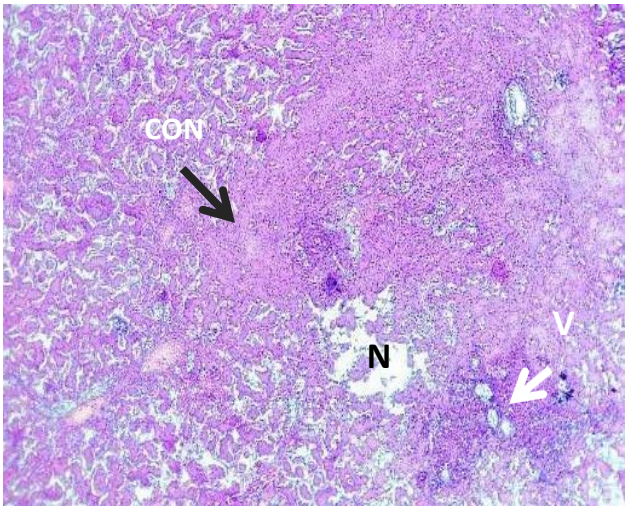


Figure (14) shows a tissue section of chicks liver infected with chronic respiratory disease, noting the congestion of the hepatic portal vein(HPV) and the gathering of inflammatory cells around the hepatic artery(HA) and bile duct (B).(H&E).(400X).

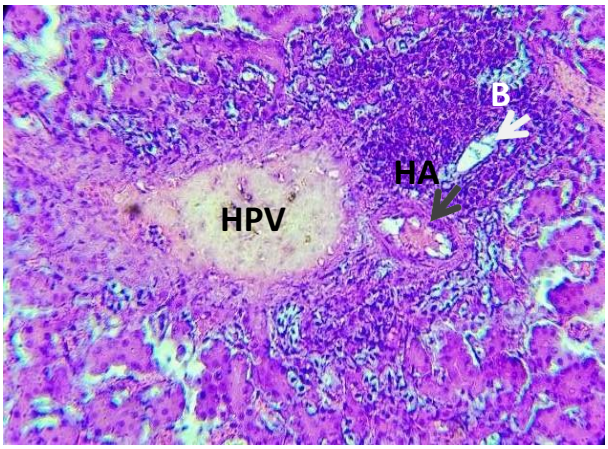


Figure (15) shows a histological section of the heart for chicks without chronic respiratory disease, showing the Cardiac myocyte.(H&E).(400X).

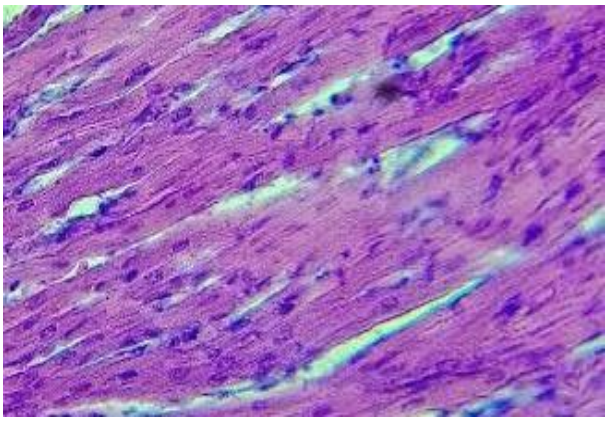


Figure (16) shows a histological section of the heart for chicks without chronic respiratory disease, showing the pericardium(PC) (H&E).(400X).

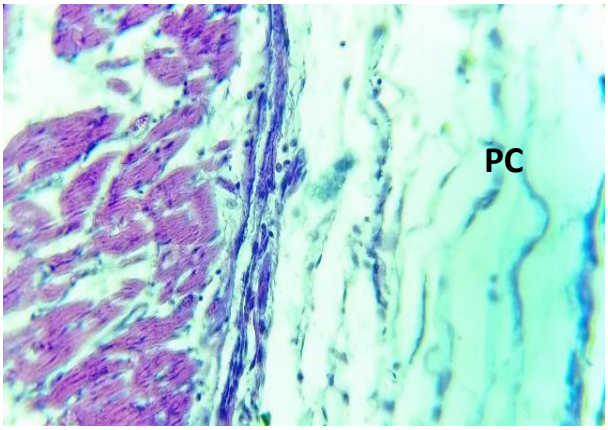


Figure (17) shows a tissue section of chicks heart with chronic respiratory disease, where inflammatory cells (arrow) accumulate in the pericardium(PC) and between myocytes(MC). (H&E).(400X).

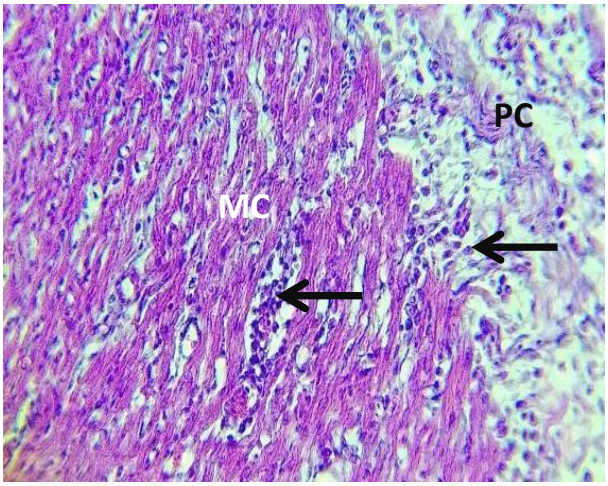


Figure (18) shows a tissue section of the heart of chicks with chronic respiratory disease, noting congestion of myocytes and bleeding(arrow). (H&E).(400X).

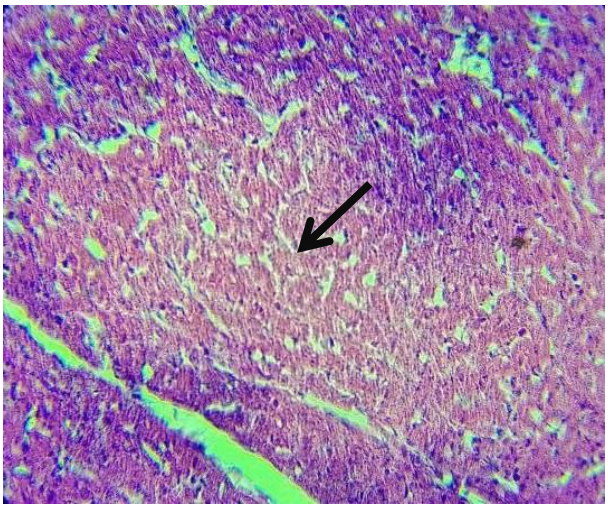


Figure (19) shows a tissue section of a chicks heart with chronic respiratory disease, noting the appearance of inflammatory cells and myocardial necrosis (arrows).(H&E).(400X).

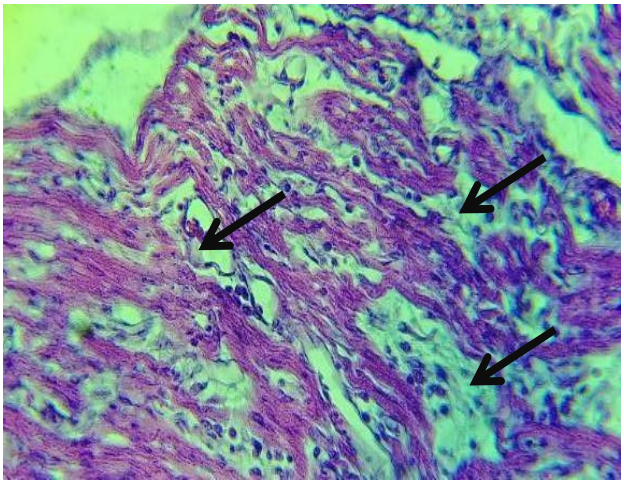
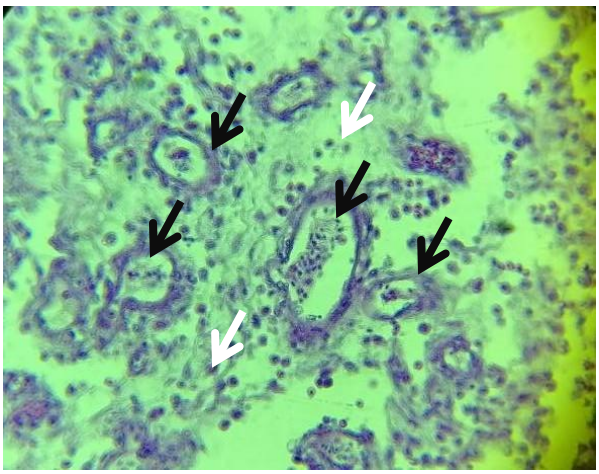


Figure (20) shows a tissue section of a chicks heart with chronic respiratory disease, noting the appearance of vacuoles in the pericardium (Black arrows) and the proliferation of inflammatory cells. (white arrows).(H&E).(400X).



Discussion

The results of the current study showed a decrease in the number of red blood cells (RBC), the rate of hemoglobin (Hb), and hematocrit(Ht), and this is due to the damage caused by mycoplasma in the respiratory tract and lungs, such as Presence of a blood clot in the capillary blood vessels of the tissues, lysis of blood cells, or hemorrhage, congestion of blood vessels, and necrosis in the lung and respiratory tract (Al-Hialli and Al-Hamdani, 2009). As mycoplasma colonizes the trachea, alveoli, and lung, which leads to hemorrhagic secretions in the lung, nasal passages, and trachea, and complications increase with E.coli bacteria, which cause perihepatitis (Gondal etal., 2015), Mycoplasma is present inside and on the surface of red blood cells (Vogl etal., 2008).It also affects an antioxidant such as glutathione in the heart (Vitula etal.,2011). It is likely to cause damage to the cells and thus decrease their numbers. The results also indicated an increase in the number of white blood cells (WBC) and may be attributed to the nutrition system for rapid growth, lack of ventilation and temperature change, which leads to a change in physiological processes, organs and tissues, which increases the adaptive defense processes with an emergency increase that leads to the occurrence of lesions or pathological changes in these tissues (Bains , 2005). Or it may be due to infection with one of the common respiratory diseases, such as the case of infection with E. coli, which is one of the naturally occurring germs, but it turns into a pathogen after the chicks are exposed to stress as a result of an increase in ammonia. (Jordan etal., 2002). It may be attributed to the destruction of the cilia of the epithelial

cells and their dissociation as a result of non-pathogenic factors, which leads to exposing the cells to bacterial invasion (E.coli). (Ginns etal., 2000) and then an increase in white blood cells. The results also indicated an increase in the value of liver enzymes ((AST) (ALT) and cardiac protein Troponin(Tn).so The results of the histological examination of the liver and

heart also showed the presence of inflammation, congestion, necrosis and vasculature, In birds, chronic respiratory disease (CRD) causes inflammation of the liver and peripheral fibrosis, as well as inflammation of the pericardial cavity of the heart. (Charlton etal., 1996 and Yamamoto, 1991). the mycoplasma germ spreads throughout the body, as it was isolated from the internal organs such as the liver, heart, spleen and kidneys (Much etal., 2002), Mycoplasma also affects antioxidants in all internal organs, including the liver, heart, spleen, lungs, and plasma (Vitula etal., 2011). Cells are damaged. such as heart muscle (Fudge etal., 1997), due to increased oxidative stress. Mycoplasma infection is associated with oxidative damage to cells and tissues of the host body due to reactive oxygen species generated by both the immune system and the host system and bacteria, which is the factor of its virulence (Almagor etal., 1983 and Sun etal., 2008). The damage may also be due to E. coli bacteria, as M. gallisepticum causes damage to the epithelial tissues in the upper respiratory tract of the bird, which exposes it to secondary bacterial infection Opportunistic infections such as pathogenic E.coli and other enteric bacteria can infiltrate the gastrointestinal tract and infect many organs, producing potent endotoxins and causing septicemia, which in turn leads to organ failure and death (Stipkovits and, Kempf, 1996). E.Coli bacteria may cause pericarditis and liver necrosis in addition to pneumonia (Cunha etal, 2017and Borgesa etal., 2017).this explains the high level of enzyme (AST, ALT, Tn), As the increase in the level of ALT and AST enzyme is the occurrence of inflammation or injury in the liver (Raulf etal., 1985, Wei etal. 2015). As for the Troponin protein, its high level indicates damage to the heart muscle cells, such as inflammation and necrosis, and its level rises directly with the degree of damage. (Chaulin, 2021; Thygesen etal., 2018). Histological sections of the liver and heart of infected chickens showed inflammation, congestion, necrosis, and vasculature. Both mentioned (Charlton etal., (1996) and Yamamoto, (1991). that chronic respiratory disease in birds causes inflammation in the pericardium and liver, and this is consistent with the findings of the current research.

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