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THE ROLE OF HISTOPATHOLOGY IN DIAGNOSIS OF FOWL ADENOVIRAL INFECTION IN BROILER CHICKENS- A CASE STUDY

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ABSTRACT: Adenoviral infection induced many diseases in chickens and wild animals. The histopathology used as a diagnostic tool for diagnosis of adenoviruses. Nineteen broiler chickens(Ross308/2-3 weeks)) deaths were collected by the owner flocks , the veterinarian made a necropsy to the recent dead cases and sent to the pathology lab., Department of Pathology, College of Medicine, Qassim University, KSA. The routine vaccination done for both Marek's and IBD via (avinew + newovac(trt) + gumboro + vit (ad3e+b.k.) + boxygint).

The prominent clinical signs of the diseased chickens, mentioned by the owners) were lethergy, depression, ruffling feathers, hudding and unable to move .At necropsy, liver,(kidney and heart) displayed swollen, pale yellow, friable and depressed necrotic foci surrounded by red zone. Redding with round edges and multifocal hemorrhagic area also observed. The histopathological findings were hepatocytic hydropic and fatty degeneration with coagulative necrosis with lymphoplasmacytic infiltrations and hemosiderosis. The pathognomonic intranuclear inclusion bodies (eosinophilic in 5 cases and basophilic in 14 cases) in the degenerated hepatocytes were also detected. We can conclude that Postmortem examination as well as the microscopic findings revealed hepatitis with basophilic and eosinophilic intranuclear inclusion bodies in the degenerated and necrotic hepatocytes due to adenoviral-like inclusion body hepatitis.

Keywords: Adenovirus-Histopathology-Broiler Chickens- Necrosis-Hepatitis-Diagnosis

INTRODUCTION

The main functions of the liver of chickens are storing and transpiration of the digestaj, besides its role in the elimination of the waste products. Viral hepatitis is a disease caused by adenoviruses, avian hepatitis E virus (HEV), duck hepatitis virus (DHV), and turkey hepatitis virus (THV) (Al-Sheikhly and Mutalib, 1979 and Horwilz, 1995). The diseases flock had pale, swollen, friable and hemorrhagic liver with pathognomonic histological changes, including basophilic nuclear inclusion bodies the hepatocytes, (Younus et al., 2017). They are medium-sized (60-90 nm), non-enveloped icosahedral viruses containing double-stranded DNA, (Foy, 1997). Adeno- viruses have a worldwide distribution and are responsible for latent adverse infections, depending upon viral tropism, (McCracken and Adair, 1993). Naturally avian denovirus infections in poultry may be associated with hepatitis, splenitis, enteritis, ventriculitis, pancreatitis, or altered reproduction (egg drop syndrome), (Kenneth et al., 1998). Adenoviruses spread via respiratory and fecal particles and can withstand many adverse physical and chemical conditions outside the body and water (American Academy of Pediatrics, 1997). Several mortalities occurred in broiler chickens in Iraq (from 1997-1998), suspected occurred due to IBH disease. The disease was seen mainly in 4-6 weeks old broiler chickens .The most common gross findings were petechial hemorrhagic areas in enlarged fatty liver. The affected hepatocytes showed fatty change, intranuclear inclusion bodies in the degenerated hepatocytes (eosinophilic in most cases and basophilic in five cases) (Al-Sheikhly and Mutalib, 1979). In south Canada, young broiler (10-14 day) mortalities recorded .The birds suffered severe respiratory distress , depression , presence of food in croup and finally , showed dead. Postmortem examination revealed friable, pale yellow -white livers scattered with hemorrhages. Coagulative-type of necrosis with bluish nuclear viral aggregates showed in the diseased hepatocytes (Philippe et al., 2005). IBH is diagnosed in 12-day -old broiler chickens with history of high mortalities. At necropsy, the liver showed pale, mottled with reticulated pattern with subcapsular minutes of hemorrhage, clear serous exudates accumulated in the pericardial sac and decreased size of bursa of fabricators. The histopathological examination of liver paraffin sections revealed diffused areas of necrosis associated with mixed inflammatory cell infiltration and large basophilic I/N inclusion bodies for IBH. IBH caused by Group I avian adenoviruses (Gabriel, 2008 and Morad et al., 2015). Genetic phenotyping of three fowl adenoviruses (FADV) induced IBH and HP syndrome in China were discussed and Postmortem changes represented by severe hepatitis and hydropericardium .In Damanhur city, Egypt. FAdV was on chicken embryonic liver cells was detected by conventional PCR targeting conserved region in hexone gene (Mohamed et al., 2018). Grossly, swollen, friable liver with round edges and Vacuolar; with MNC infiltration in the hepatocytes with intranuclear inclusions detected proving that the viral cause is FAdV and the disease is IBH. Loss of normal architecture of the hepatocytes with coagulative necrosis and IN inclusion bodies .Postmortem examination and microscopic examination showed severe hepatitis, associated with basophilic inclusions, splenitis, myocarditis and nephritis (Asok et al., 2018 and

Eltholohetal, 2019). The present investigation was aimed to detect the cause of mortalities among broilers chickens by using the histopathological technique for diagnosis of adenovirus -like infection that suspected causing IBH disease.

II-MATERIALS AND METHODS

Liver(kidney and heart collected also but did not showed any characteristic changes) samples from Nineteen broiler chickens were collected and fixed in 10% neutral buffered formalin then processed as usual routine histopathological processes the Paraffin sections (4 µm thick) were cut by sliding microtome and stained with Hematoxylin and Eosin for histopathological studies according to (Bancroft and Stevens, 1975).

RESULTS AND DISCUSSION:

The prominent clinical signs in diseased birds were lethargy, depression, ruffling feathers; raised mortalities and birds are unable to move (Morad et al., 2015; Houssam et al., 2017 and Oliver-Ferrano et al., 2017). At necropsy, livers were swollen, pale or of yellowish-brown discoloration. Friable and depressed necrotic foci that surrounded by hyperemic zone. Some other cases were congested with multiple petechial hemorrhages on the surfaces (Fig.1-4). Our results were coincide with those reported by (Alsheikhly and Mutalib, 1979; Pedro, 1995; McFeren and Smyth, 2000; Philippe et al., 2005; Gabriel, 2008; Kumar et al., 2013; Morad et al., 2015; Zhao et al., 2015; Miguel et al., 2016; Revajova et al., 2017; Mohamed et al., 2018; Asok et al., 2018 and Eltholoh et al., 2019).

Microscopic examination of the liver revealed severe degenerative as well as necrotic changes of the hepatocytes represented by hydropic degeneration, fatty change and coagulative necrosis that admixed with mononuclear cells infiltration. Congestion, hemorrhage, hemosiderosis with kupffer cells hyperactivation were also, noticed. Intranuclear Inclusion Bodies were also noticed (esinophilic/basophilic) (Fig.5-8). Nearly the same results were recorded by (McCracken et al., 1976; Al-Sheikhly and Mutalib, 1979; Pedro, 1995; Philippe et al., 2005; Gabriel, 2008; Dolka et al., 2012; Kumar et al., 2013; Morad et al., 2015; Zhao et al., 2015; Miguel et al., 2017; Schachner et al., 2016; Revajova et al., 2017; Morshed et al., 2017; Mohamed et al., 2018; Asok et al., 2018 and Eltholoh et al., 2019).

CONCLUSION

Postmortem examination and microscopic findings revealed hepatitis with basophilic and esinophilic intranuclear inclusion bodies in the degenerated and necrotic hepatocytes. Based on histopathological analysis and the previous researchers, the mortality in this flock was due to adenoviral-like inclusion body hepatitis.

Table (1): Showed lesion score and severity.

Lesion	Score
Hepatitis	++(16 cases)
Necrosis	+(10 cases)
Vacuolar and hydropic degeneration	++(19 cases)
IB(Basophilic)	++(14 cases)
IB(Esinophilic)	+(5 cases)

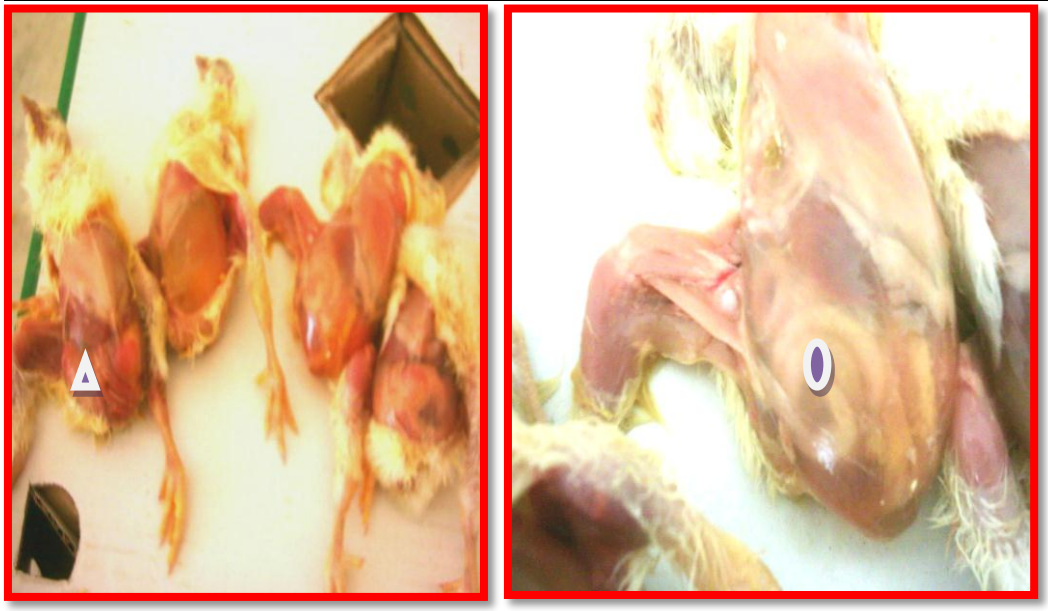

Fig. (1&2): Chicks died due to Adenovirus: Abdominal ascites (sympol), pale yellow and necrotic liver (Arrow head).

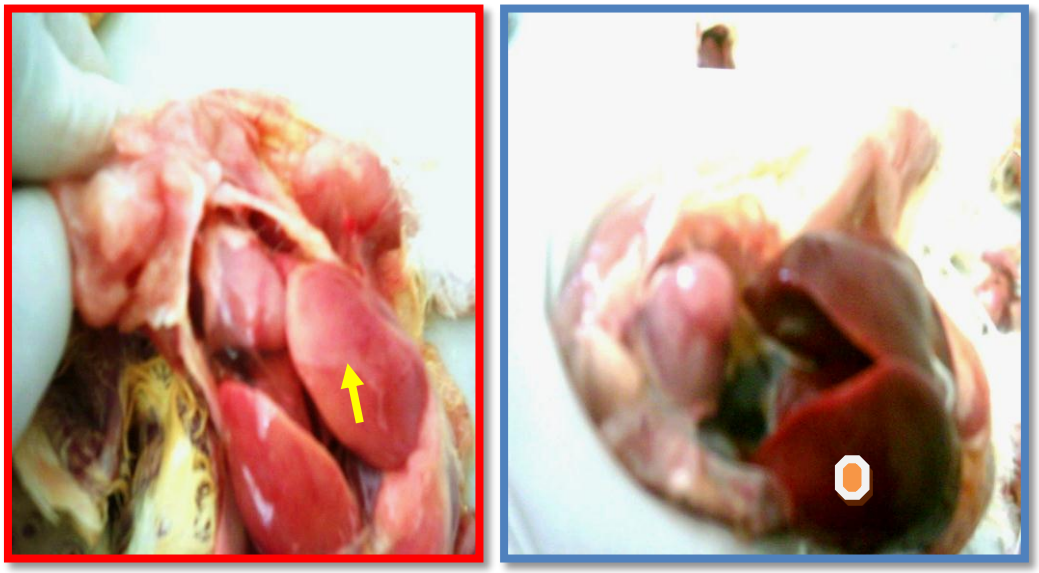

Fig. (3&4): Pale yellow, hemorrhagic (Astrix) and Necrotic liver (Arrow).

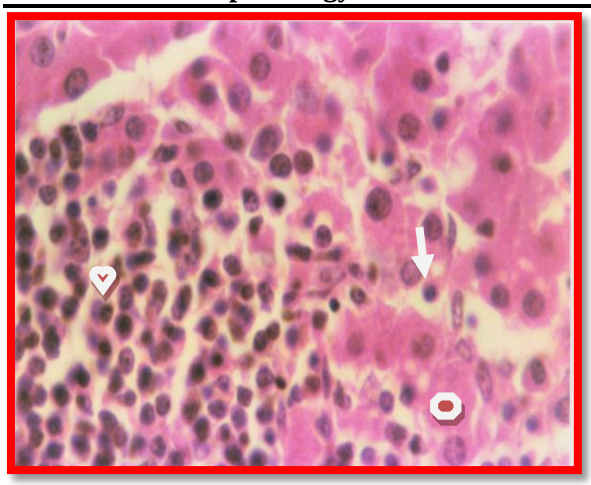

Fig. (5): Liver of Broiler chicks dead due to adenoviral infection showing: Multifocal hepatocytic necrosis (sympol), kupffer cells hyperactivation, hemosiderosis (heart sympol), MNC infiltration (Interface hepatitis) and (I/N) basophilic inclusion bodies (arrows). H&E.X.1000.

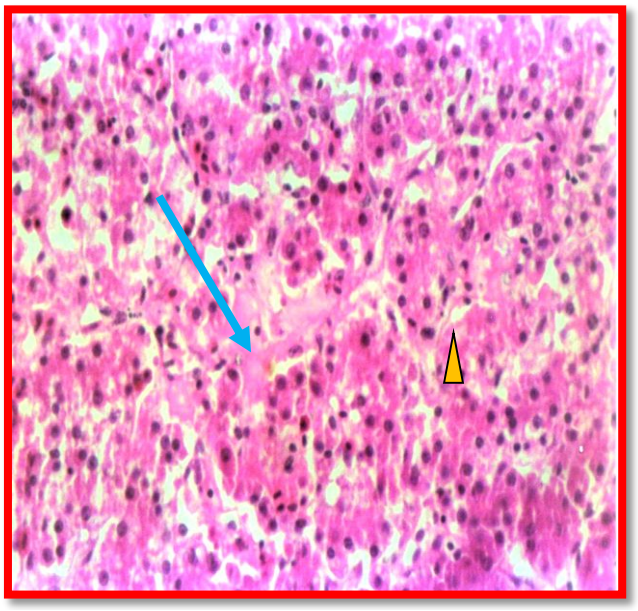

Fig. (6): Hepatitis and necrosis (arrow + astrix) (N) with diffuse MNC infiltrations (H&E , X.200)

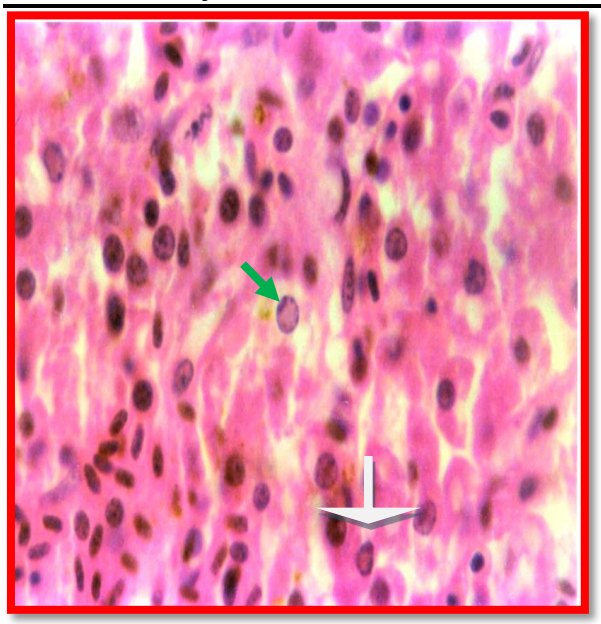

Fig. (7): Necrosis, hemosiderosis, hemorrhage, and I/N inclusions (esinophilic / smudgy (clearly soft) nuclear inclusion.

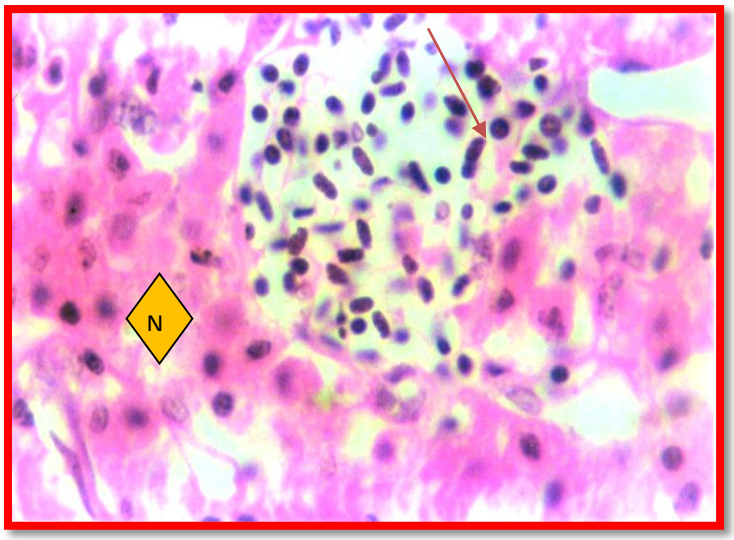

Fig. (8): Necrosis and I/N smudgy nuclear inclusions bodies, H&E. X. 400.

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الملخص العربى

دور التشريح المرضي في تشخيص عدوى الفيروس الادينو في دجاج التسمين ـ دراسة حالة

السيد الديب مهنا و محمد عبد الباري مندور

الخلفية العلمية :تسبب عدوى الفيروس الادينو العديد من الأمراض في الدواجن والحيوانات البرية.
يستخدم علم التشريح المرضي كأداة تشخيصية لتشخيص فيروسات الأدينو ، وخاصة في الشرائح الزجاجية المقطوعة بالبارافين والمصبوغة.
أهداف الدراسة: الهدف من الدراسة هو اكتشاف سبب الوفيات (الوفيات) في مزرعة دجاج التسمين ، بريدة ، القصيم ، المملكة العربية السعودية.
المواد والطرق البحث: تم جمع ما مجموعه 19 وفاة من دجاج اللاحم وأرسلت إلى مختبر الأمراض ، قسم علم الأمراض ، كلية الطب ، جامعة القصيم ، المملكة العربية السعودية. تم جمع عينات من الكبد ووضعها في 10٪ من الفورمالين لمدة 48 ساعة عند درجة حرارة الغرفة وتم معالجتها باستخدام H&Eالطريقة الروتينية (الهيستوباثولوجى) للكشف عن التغيرات النسيجية للعدوى الفيروسة الادينو(الغدى) باستخدام المجهر الضوئي..
النتائج كانت العلامات المرضية البارزة للدجاج المصاب هي الخمول ، والاكتئاب ، والريش المتراكم ، والتكتل وعدم القدرة على الحركة. في التشريح المرضى لاحظنا وجود بالكبد بؤرًا منتفخة وأصفر باهتًا ومتفتتًا ومكتئبًا محاطًا بمنطقة شديدة الشدة. احتقان مع حواف مستديرة وبؤر النزفية لاحظت أيضا. ومثلت التغيرات الهستوباثولوجية المكتشفة عن طريق التنكس المائي للكبد ، والتغيير الدهني ونوع التخثر من النخر الذي يضاف إلى ارتشحات من الخلايا اللمفاوية وتسمم الدم. كما تم الكشف عن الهيئات المشتملة.Inclusion bodies على التضمين النووي النووي (الحمضية في خمس حالات والبازلوفيل في 14 حالة) في خلايا الكبد المتدهورة..
الخلاصة: كشف فحص ما بعد الوفاة وكذلك النتائج المجهرية عن التهاب الكبد مع هيئات التضمين النووي(الاجسام الفيروسية القاعدية والايوزينية في خلايا الكبد المتدهورة والنخرية. بناءً على تحليل الأنسجة ، فحص ما بعد الوفاة ونتائج الدراسات السابقة ، كان معدل الوفيات في هذه القطيع (الحالات) بسبب التهاب الكبد الوبائي الادينو فيروس.