

POSSIBILITY OF ISOLATION OF INCLUSION BODY OF HEPATITIS VIRUS IN LAYER HENS

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ABSTRACT

The aim of this study was isolation of the inclusion body hepatitis virus (IBH) from infected commercial layer hens in Ninevah governorate and identification the pathological effects of this virus. The natural infection of IBH revealed pathognomic basophilic and eosinophilic intranuclear inclusion bodies in the nuclei of many hepatocytes of infected chickens. Haemagglutination test of the allantoic fluid show titer 3Log_2 of the isolated virus after one passage in the chicken embryos with 1% rats washed RBCs. The experimental infection is done by injection of layers and breeders at 20 weeks age by liver homogenate from infected chickens and revealed the same pathognomic gross and histopathological lesions.

إمكانية عزل فيروس التهاب الكبد ذو الأجسام الأشمالية من الدجاج البياض

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الخلاصة

الهدف من هذه الدراسة عزل الفيروس المسبب لمرض التهاب الكبد ذو الأجسام الأشمالية من أحد الحقول للدجاج البياض في محافظة نينوى كذلك دراسة التأثيرات المرضية لهذا الفيروس. المقاطع النسجية للإصابة الطبيعية بمرض التهاب الكبد ذو الأجسام الأشمالية أظهرت آفات واصمة متمثلة بالأجسام الأشمالية القاعدية والحمضية وهي أنوية الخلايا الكبدية للدجاج المصاب. فيما أظهرت نتيجة اختبار التلازن الدموي الذي أجري على السائل الغانقي معيار 3Log_2 للفيروس المعزول بعد تمريره واحدة في أجنة بيض الدجاج باستخدام 1% كريات الدم الحمراء المغسولة للجرذان. تم إحداث الإصابة التجريبية وذلك

بحقن دجاج بعمر 20 أسبوع بمستخلص كبد للدجاج مصاب، أظهر للدجاج المحقون آثاراً مماثلة للإصابة الطبيعية.

INTRODUCTION

Inclusion body hepatitis (IBH), an economically important disease of commercial chickens, was first reported in the United State of America in 1963 as a necrotizing hepatitis in 7-week-old chickens associated with Cowdry type A intranuclear inclusion bodies in hepatocytes (1). Inclusion body hepatitis is characterized by sudden onset of mortality peaking after 3-4 days and usually stopping on day 5 but occasionally continuing for 2-3 wk, morbidity is low; sick birds adopt a crouching position with ruffled feathers and die within 48 hr or recover. Mortality may reach 10% and occasionally as high as 30%. It is normally in meat-producing birds as young as 7 days(2)and as old as 20 week(3). Group 1 avian adenoviruses or fowl adenoviruses (FAV; 12 serotypes) are associated with or incriminated in outbreaks of IBH (4, 5). The main lesions are pale, friable, swollen livers, petechial or ecchymotic hemorrhages may be present in the liver and skeletal muscle (6, 7). There are inclusion bodies in the hepatocytes. These inclusions can be eosinophilic, large, round or irregularly shaped with clear pale halo (8) or occasionally basophilic inclusions (6, 9). Embryonated eggs are insensitive for primary isolation of most serotypes of adenoviruses, and the yolk sac is a sensitive route for isolating laboratory strains (10).

The aim of this study was isolation and identification of field IBH virus with field and experimental pathological studies in affected chickens.

MATERIALS AND METHODS

Samples (livers) for virus isolation are obtained from newly dead and killed chickens which suspect infected with disease, depending on the post mortem changes and pathognomic lesion that appears on the livers of the diseased chickens. Then the treating of samples have done according to procedure that described by (11). Broiler chicken embryos are used at 9-12 day after cultivation for its inoculation with suspected fluid. the inoculation has done in allantoic sac (0.2ml/ egg). The death embryos after 24 hrs are discarded. The examination of eggs continued for 7 days, then the remaining eggs are put in the refrigerator for 4 hrs, opening eggs and collecting of the allantoic fluid under sterile condition for haemagglutination test, embryos and chorioallantoic membrane are extracted to show if there are any changes or it (12). The experimental infection is done by intraperitoneal injection of layers and breeders at 28 weeks age by liver homogenate (13), the clinical signs and post mortem changes are recorded for showing the macroscopic and microscopic lesions on the affected organs. Haemagglutination test is done to show the ability of the viruses to agglutinate the washed rats RBCs (1%) (14). Histopathological studies of liver lesions were performed by fixing affected portions in 10% neutral buffered formalin. Fixed tissues were trimmed, embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin stain. Tissue sections were then examined microscopically.

RESULTS

Natural infection with adenovirus of the commercial layer hens flock at 44 weeks age in Ninevah governorate: clinical signs of infected birds included drowsiness, ruffled feathers, decreased food intake, with the mortality 5-20%. The major post mortem finding of field infection was swollen, yellow brown colored liver with necrotic foci, in some cases pinpoint hemorrhage (Figure 1). Enlarged and marbled necrotic spleen, enlargement and paleness of kidney, regression of the ovary (Figure 2). Histopathological examination of the liver from dead and killed infected chickens showed massive liver lesions with vacuoles degenerative changes (hepatosis) and multifocal areas necrosis in the hepatic parenchyma, nuclei of many hepatocytes contain intranuclear inclusion bodies (Figure 3). In addition to congestion of central veins, associated with infiltration of inflammatory cells especially mononuclear cells in the hepatic tissue.

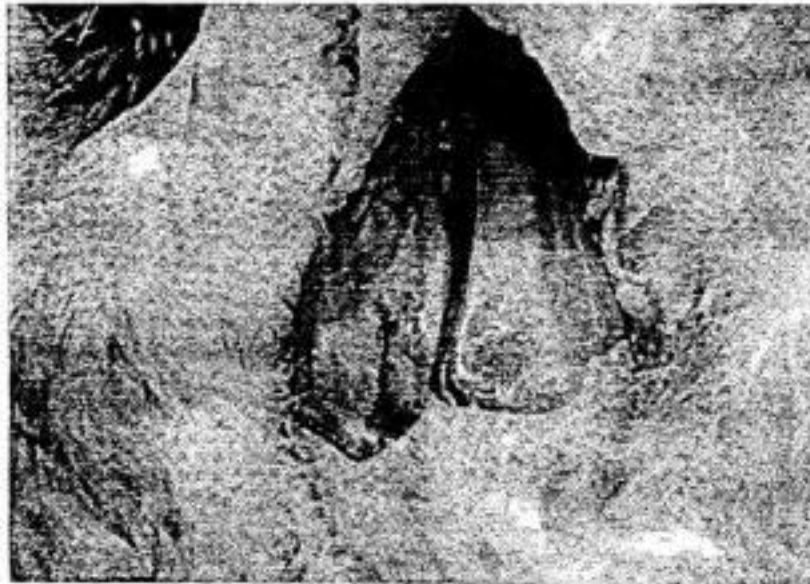


Figure 1: Liver of chicken naturally infected with adenovirus shows swollen yellow brown colored associated with multifocal necrotic foci areas.



Figure 2: Visceral organs of chicken naturally infected with adenovirus shows necrotic foci in the spleen associated with enlarged and pale Kidney and regression of the ovary.

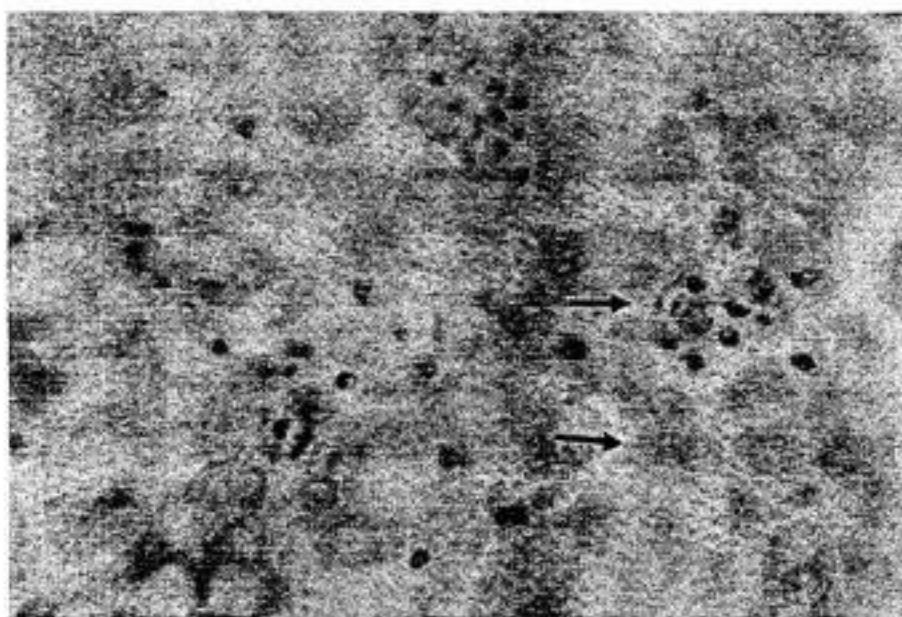


Figure 3: Histomicrograph of liver in natural infected chickens shows vacuolar degeneration of hepatocytes associated with intranuclear inclusion bodies. H&E stain, X100.

Embryonated chicken eggs: the result of inoculation in chicken embryos showed dwarfism and hemorrhage on the embryos compare with control, in another side the chorioallantoic membrane show thickness and hemorrhage. Haemagglutination test of

the allantoic fluid show titer 3Log_2 of the isolated virus after one passage in the chicken embryos with 1% rats RBCs.

Experimental infection: the affected birds show anorexia, dullness, and ruffled feathers. Gross lesions revealed the same lesions of livers, spleen, kidneys to the lesions of the field infection in addition to accumulation of 1-2 ml pericardial fluid (Figure 4, 5). Histopathological examination of the livers revealed the same lesions of livers of the natural infection, except in natural infection showed larger basophilic intranuclear inclusion bodies in the nuclei of hepatocytes (Figure 6).



Figure 4: Liver of chicken experimentally infected with adenovirus shows swollen liver associated with necrotic foci associated with mild accumulation of pericardial fluid.



Figure 5: Visceral organs of chicken experimentally infected with adenovirus shows enlarged pale kidney with necrotic foci on the spleen and regression of the ovary.

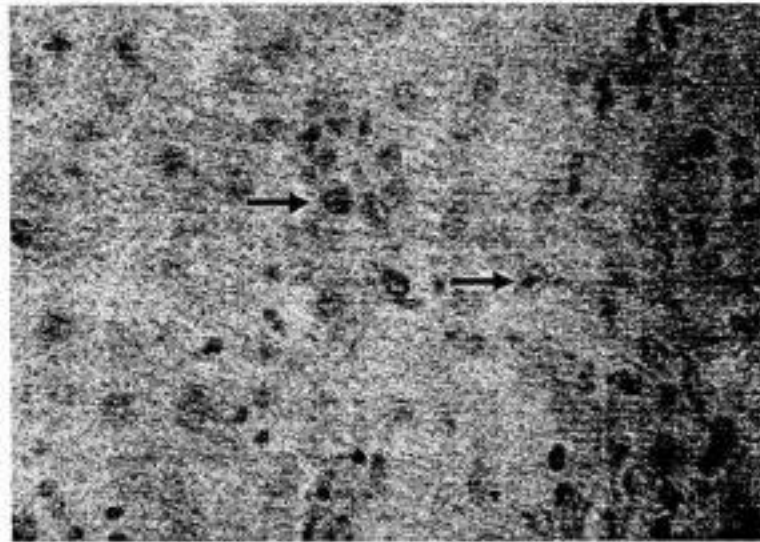


Figure 6: Histomicrograph of liver in experimentally chickens infected with adenovirus shows vacuolar degeneration of hepatocytes associated with intranuclear inclusion bodies .H&E stain.X100.

DISCUSSION

Avian adenoviruses are present in most commercial poultry and many other avian species. Our study included isolation of adenovirus from natural infection with adenovirus of the commercial layer hens flock at 44 weeks age in Ninevah governorate and confirm the isolation by identification the pathognomic inclusion bodies hepatocytes with pathological changes in liver and other organs (6-9) with mortality 5-20% (2) at 44 weeks age (3). The embryonated chicken eggs showed susceptibility to isolated virus as result of dwarfism and hemorrhage and thickening of chorioallantoic membrane (15), this lesion occur due to the disturbance in the respiratory function of embryo after the infection of CAM that considered the main organ of respiration in embryonated chicken eggs (16). Positive result of agglutination test with rat RBCs only and this result give a good indicator that the isolated virus is adenovirus (1).

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