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PATHOLOGICAL AND MOLECULAR INVESTIGATION OF DUCK VIRAL HEPATITIS (DVH) IN KALIOBEYA GOVERNORATE, EGYPT

***Nora M. Elseddawy and Heba M. Abdel-Ghany**

*Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Elzera'a square, 44511 Sharkia, Egypt.

***Corresponding Author: Nora M. Elseddawy**

Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Elzera'a square, 44511 Sharkia, Egypt.

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ABSTRACT

One thousand, 14 day-old Pekin ducklings suffered from depression, eye closing, watery diarrhea and the affected bird fall on their side and kick spasmodically with head stretched upward and backward (opsithotonos position) with mortality rate reached up to 80% reared in kaliobeya governorate were the subject of our investigation. Specimens from different organs including liver spleen, lungs, intestine, heart, gizzard, proventriculus, pancreas, brain and bursa were collected and subjected to the pathology The collected specimen were fixed in 10% neutral buffered formalin solution, dehydrated in gradual alcohol (70-100%), cleared in xylene and embedded in paraffin. Five microns thickness paraffin sections were prepared and stained with hematoxylin and eosin (HE) dyes then examined microscopically. Some of the collected specimens were stored frozen at -20°C to be subjected Reverse - transcriptase polymerase chain reaction (RT-PCR) for the detection of Duck viral hepatitis (DVH). Macroscopically, the liver was enlarged, yellowish brown in color with hemorrhagic spots on its surface, the kidneys, spleen and lungs were enlarged and congested. The brain showed congestion in meninges with hemorrhagic patches beside congestion of intestinal mesentery was observed. Hemorrhage, congestion, cell swelling, necrosis with infiltration of heterophiles and lymphocytes were observed microscopically in the examined organs. PCR technique was positive for Duck viral hepatitis and it was the best tool to diagnose the disease.

KEYWORDS: DVH, poultry, Picornaviridae, opsithotonos, PCR.

INTRODUCTION

Viral hepatitis in poultry is a complex disease caused by several viruses belonging to different families including avian hepatitis E virus (HEV), duck hepatitis virus (DHV), fowl adenoviruses (FAdV) and turkey hepatitis virus (THV).[1]

The disease firstly described in New York, in 1949. DHV outbreaks were reported in Germany, Japan, England and Canada.[2]

Duck viral hepatitis (DVH), an infectious disease that affects ducklings within 3 weeks of age and caused by duck hepatitis viruses (DHVs) belonging to Picornaviridae and Astroviridae families. DHV was originally divided into three serotypes: type 1 (DHV-1), type 2 (DHV-2) and type 3 (DHV-3) DHV-1 belongs to the Picornaviridae family and it is the most virulent, common serotype that spread worldwide, while DHV-2 and DHV-3 belong to the Astroviridae family.[3]

DVH is an acute, contagious, rapidly spreading disease and highly fatal for ducklings within three weeks. The disease causes highly losses in duck farms due to increase rate of mortality which reached up to 80% and morbidity 100%.[4]

DHV-1 may cause high mortality in 1-week-old ducklings but adult ducks have strong resistance to DHV-1 infection. The hepatocellular injury in is caused by activated inflammatory factories not by the damaging agent.[5]

The DVH may be transmitted by parental or oral routes. The virus is shedding in faeces and is transmitted through direct contact between birds or by fomites such as feed, water, equipment and brooders. But egg transmission not occurred.[6]

The typical clinical signs of DVH-1 were depression, wing dropping, eye closing, watery and greenish diarrhea, somnolence, ataxia and death occur after an hour of onset. Affected bird loses contact with main flock, fall on their side and kick spasmodically with head stretched upward and backward (opsithotonos position).[7]

The gross lesions were restricted to liver which is enlarged, fragile, yellowish brown in color with pinpoint

hemorrhage or multiple punctate and ecchymotic hemorrhages. The kidneys and spleen were swelling and hemorrhagic, congestion of lungs and cerebral meninges with mild enlargement of bursa and pancrease were seen.[8]

The microscopic lesions of DVH were restricted to liver which showed hemorrhage, cell swelling, focal steatosis and hydropic degeneration with focal point of necrosis, the necrotic hepatocytes characterized by lightly colored cytoplasm and big or small vacuole. Hyperplasia of bile duct with perivascular lymphocytic infiltration in addition to large number of granulocytes and heterophils were seen inside the liver tissue.[9] The spleen showed proliferation of reticuloendothelial cells with atrophied and necrotic lymphocytes around center arterioles leaving spherical vacuoles beside infiltration of few numbers of heterophils inside the splenic tissue. The bursa of fabricius showed desquamation and necrosis of epithelium with necrosis of lymphocytes and infiltration of few numbers of heterophils inside lymphoid follicles. The kidney showed hemorrhage and hyperemia besides desquamation of lining epithelium, small spherical vacuoles were seen inside the epithelial cells of renal tubules and heterophils infiltration. The pancrease showed spherical vacuoles inside epithelial cells with hyperplasia of lymphocytes. Mild hyperemia and hemorrhage together with congestion of blood vessels were seen in lung, heart, intestine and brain with myositis and inflammation of proventriculus.[10,11]

Diagnosis of DVH in ducklings is not depended only on the clinical findings and pathology, but confirmatory diagnosis can be occurred by Reverse - transcriptase polymerase chain reaction (RT-PCR).[12]

MATERIALS AND METHODS

Specimens

One thousand 14 day-old Pekin duckling reared in kaliobeya governorate representing 80% mortality was the subject of our investigation; affected ducklings were representing nervous signs and opisthotonos position. Specimens from different organ of 80 ducks were collected. The collecting specimens were subjected to pathology and liver from 10 ducklings pooling as one sample and subjected to PCR laboratory (stored freezing -20C). The main examined organs were liver, kidneys, spleen, lungs, intestine, heart, gizzard, proventriculus, pancreas, brain and bursa.

Pathological examination

The necropsy was occurred for detection of DVH in different tissue and visceral organs. The collected specimens were fixed in 10% neutral buffered formaline, dehydrated in alcohol (70-100%), cleared in xylene and embedded in paraffin. Five micron thickness paraffin sections were prepared and stained with hematoxyline and eosin (HE) dyes and then examined microscopically.[13]

Molecular detection of IBV by PCR

DNA extraction was performed according to QIAamp Viral RNA Mini extraction kits (Qiagen, Germany). Oligonucleotide primer / probe sequences (Metabion, Germany), 5' CCTCAGGAACTAGTCTGGA, - 3′, 5′-GGAGGTGGTGCTGAAA- 3', were selected from a previously published paper.[14] Real-time PCR was performed with a final volume of 25 µL of the following reaction mixture: 12.5 µL QuantiTect Probe RT-PCR Master Mix (2X) (Sigma, UK), 0.5 µL of each primer (50 pmole), 0.25 µL QuantiTect RT Mix, 7µL template DNA Water. Real time PCR thermal cycling was applied according to a previously published paper.[14] with some modification; reaction mixtures were incubated at 50˚C for 30 min for Reverse transcription followed by Primary Denaturation at 95˚C for 5min then Secondary Denaturation at 94˚C for 30 sec and Annealing at 50˚C for 30 sec, Extension at 72˚C for 45 sec and Final Extension at72˚C for 10 min and number of cycles were 35.[14]

RESULTS

Clinical signs

The most observed clinical signs were depression, eye closing, watery diarrhea and the affected bird fall on their side and kick spasmodically with head stretched upward and backward (opsithotonos position) and the mortality rate reached up to 80%.

PATHOLOGICAL FINDINGS

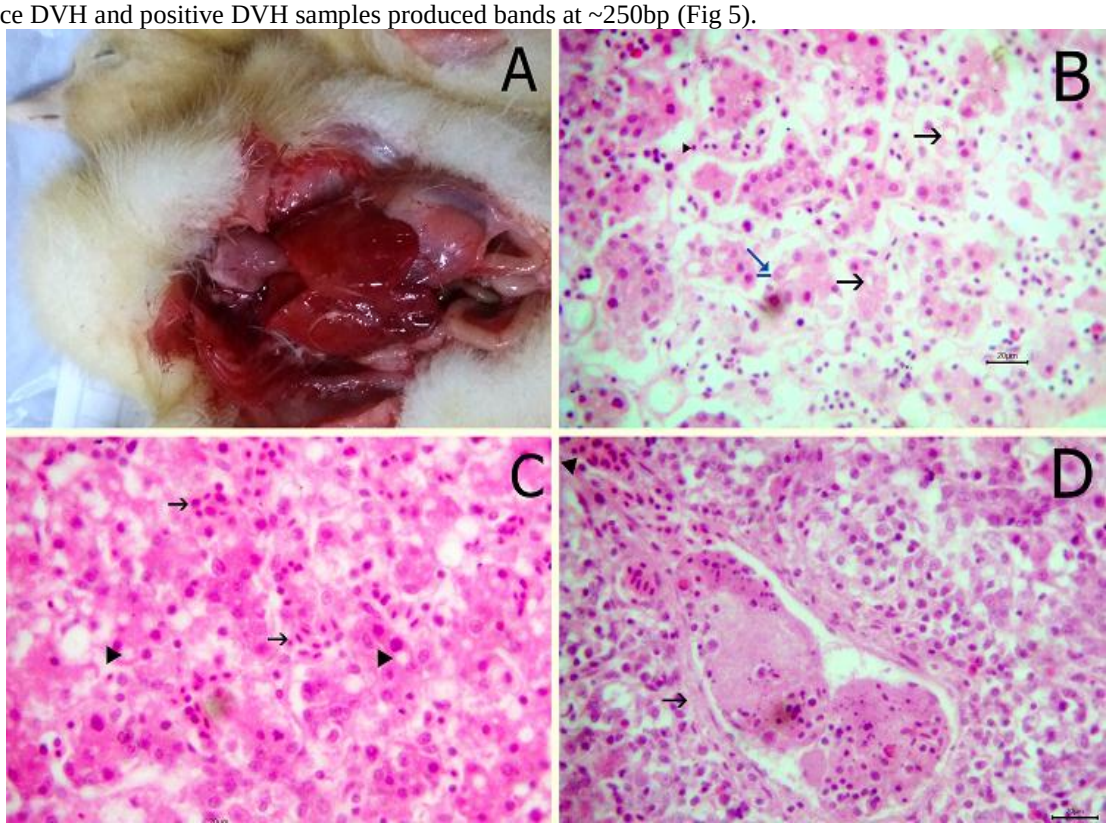
Macroscopically, the liver was enlarged, yellowish brown in color with hemorrhagic spots on its surface (Fig 1A). The kidneys, spleen and lungs were enlarged and congested. The brain showed congestion in meninges with hemorrhagic patches beside congestion of intestinal mesentery was observed.

Microscopically, the liver showed distortion of hepatic cords with many necrotic area represented by pyknotic nuclei together with small spherical vacuole inside the cytoplasm of hepatic cells were reported (Fig1B). Congestion of hepatic sinusoids was detected with extravasated erythrocytes and heterophilis infiltration (Fig1C). The blood vessel showed fibrinous thrombi with infiltration of round cells (Fig1D). The portal area showed congestion and thickening of the blood vessel with round cell infiltration together with hyperplasia of lining epithelium of bile duct and formation of newly formed bile ductless (Fig 2E). Some bile duct showed desquamation of the lining epithelium with endotheliosis and hyalinization of portal vein (Fig2F). **The bursa of fabricius** showed desquamation of epithelial mucosal cells with depletion of some lymphoid follicles (Fig2G). Interfollicular edema was also seen between lymphoid follicle with heterophilis and mononuclear cells infiltration (Fig2H). Necrosis of lymphocytes inside lymphoid follicles represented by pyknotic nuclei together with hemorrhage were detected (Fig3I). **The pancrease** showed some small spherical vacuole inside its acini (Fig3J). Necrosis of pancreatic acini and islets of

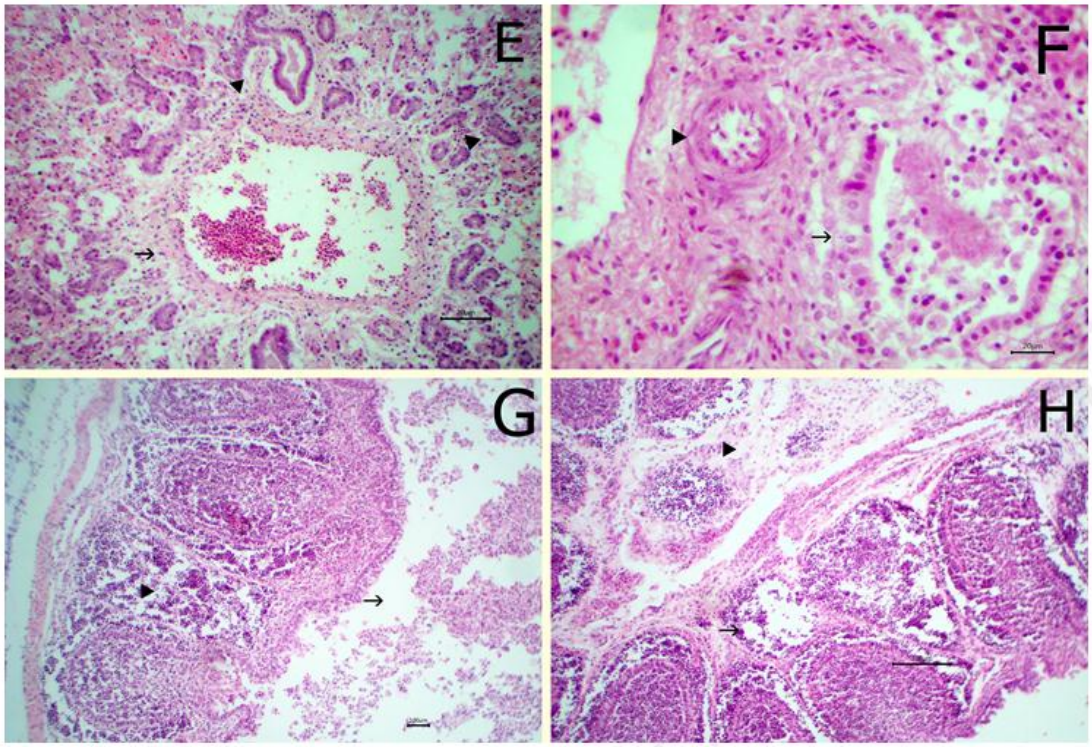
Langerhans cells represented by pyknosis and karyorrhexis of the nuclei with infiltration of heterophilis, erythrocytes and mononuclear cells infiltration were seen (Fig3K). **The kidney** showed epithelial cast inside lumen of renal tubules with small spherical vacuoles inside lining epithelium (Fig3L). Hemorrhage was noticed between renal tubules. **Heart** showed congestion with endotheliosis and hyalinization of the wall of blood vessel (Fig4M). Interstitial edema and hemorrhage were seen between muscle fiber and around blood vessels (Fig 4N). Focal aggregation of heterphilis and mononuclear cells around cardiac blood vessels was detected (Fig4O). **The lungs** showed congestion of blood vessel and thickening of interalvealar septa by hemorrhage and mononuclear cell infiltrations (Fig4P).**The spleen** showed necrosis of lymphocytes inside splenic tissue with heterophilis infiltrations (Fig4Q). **The intestine** showed catarrhal enteritis represented by desquamation of superficial layer of intestinal villi with leukocytes infiltration in sub mucosa (Fig 4R). The muscular layer showed hyalinization and mononuclear infiltrations. **The proventriculus** showed mononuclear cell infiltrations, heterophilis and hemorrhage inside the mucosa. **The Gizzard** showed necrosis of epithelial cells of mucosa with focal aggregation of mononuclear cells infiltration and heterophilis inside muscular layers. The brain showed congestion of blood vessels.

Detection and identification of DVH by using RT-PCR

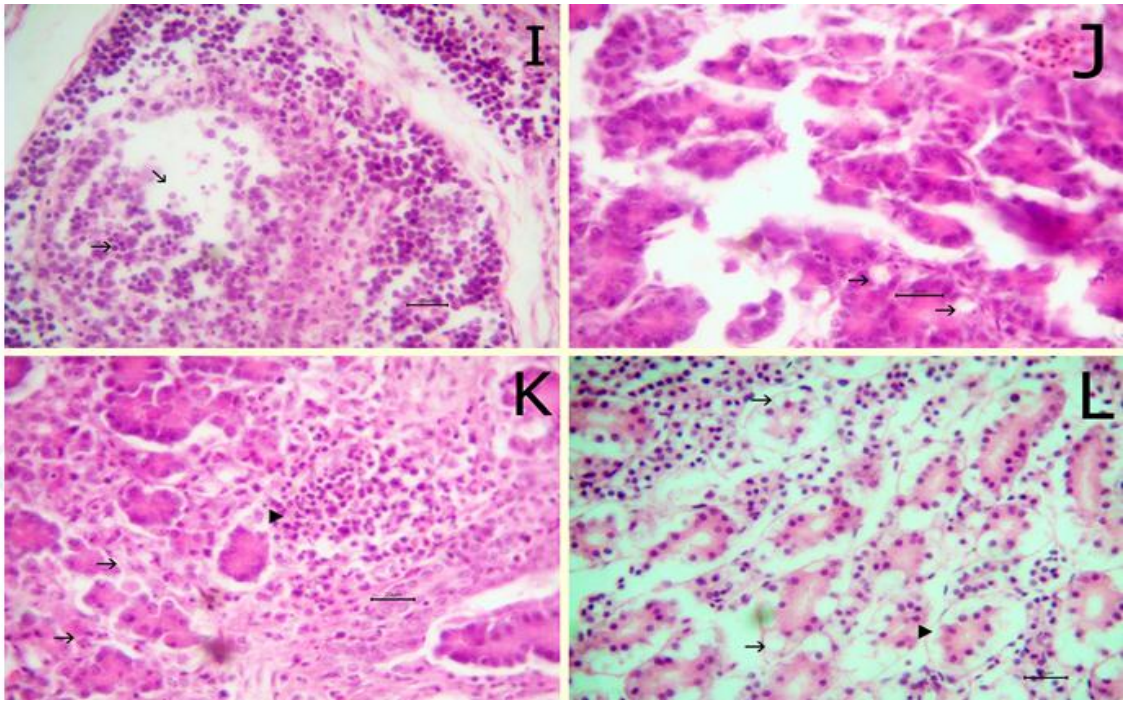
Reference DVH and positive DVH samples produced bands at ~250bp (Fig 5).



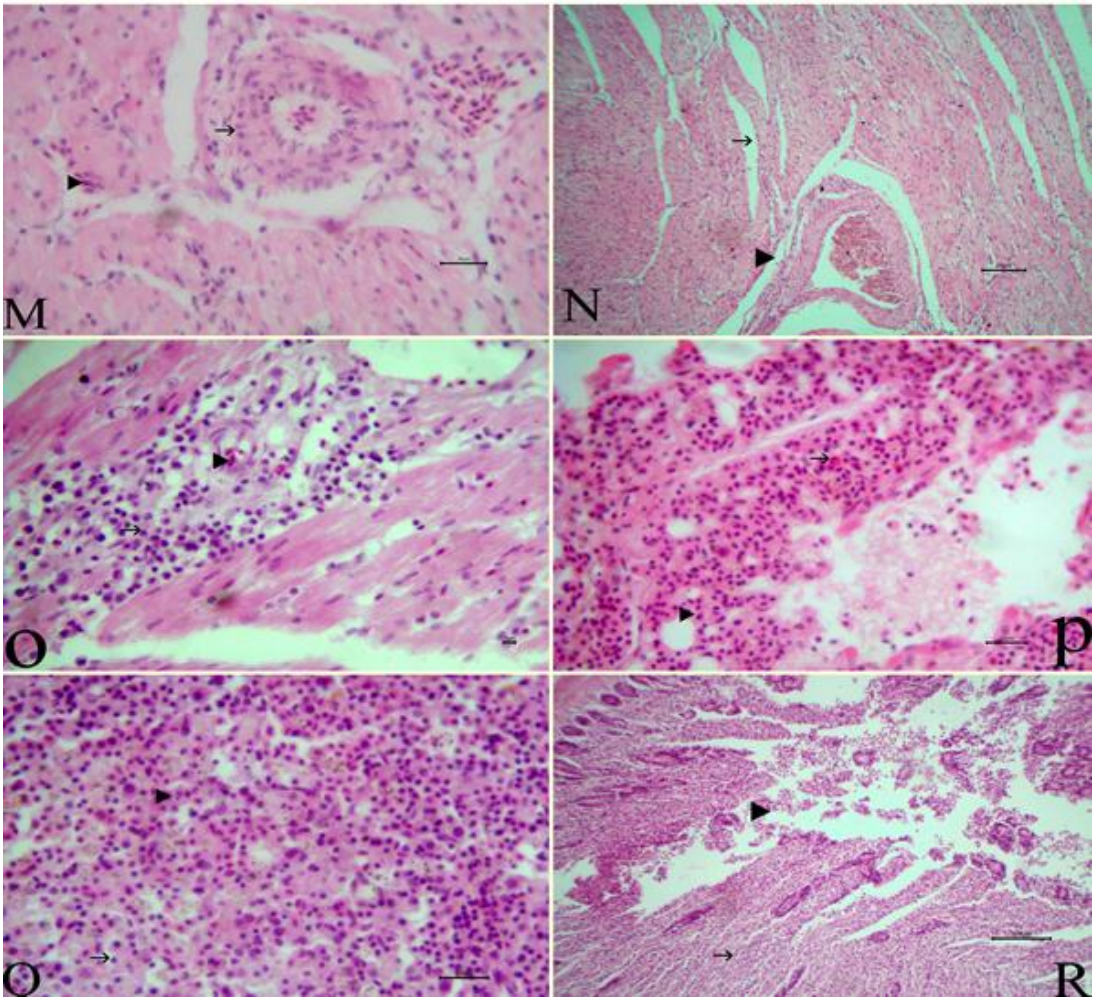
"Fig. 1", A (Dead duckling showing opsithotonos position with enlarged liver and hemorrhagic spots on its surface), B(The liver showing distortion of hepatic cords (blue line) with many necrotic area represented by pyknotic nuclei (arrow head) and small spherical vacuole inside the cytoplasm of hepatic cells (arrow) H&E, 20 bar), C(The liver showing congestion of hepatic sinusoids (arrow) with small spherical vacuole inside the cytoplasm of hepatic cells (arrow head) H&E, 20 bar), D (The portal vein showing fibrinous thrombi with infiltration of round cells (arrow) H&E, 20 bar).



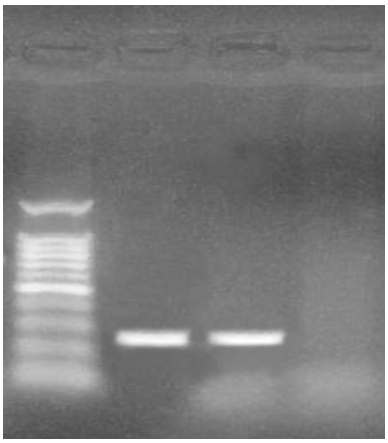
"Fig. 2", E (The portal area showing congestion and thickening of the blood vessel with round cell infiltration (arrow) with hyperplasia of lining epithelium of bile duct with newly formed bile ductules (arrow head) H&E, 20 bar), F (The bile duct showing desquamation of the lining epithelium (arrow) with endotheliosis and hyalinization of portal vein (arrow head)H&E, 20 bar), G (The bursa of fabricius showing desquamation of epithelial mucosal cells (arrow) with depletion of lymphoid follicles (arrow head)H&E, 20 bar), H (The bursa of fabricius showing Interfollicular edema between lymphoid follicles (arrow head) H&E, 100 bar).



"Fig. 3", I (The bursa of fabricius showing necrosis of lymphocytes inside lymphoid follicles represented by pyknotic nuclei (arrow) H&E, 20 bar), J (The pancrease showing small spherical vacuole inside its acini (arrow) H&E, 20 bar), K (The pancrease showing necrosis of pancreatic acini and islets of Langerhans cells (arrow) with infiltration of heterophilis, erythrocytes and mononuclear cells (arrow head) H&E, 20 bar), L (The kidney showing epithelial cast inside lumen of renal tubules (arrow head) with small spherical vacuoles inside lining epithelium (arrow)H&E, 20 bar).



"Fig. 4", M (The Heart showing hemorrhage (arrow head) with endotheliosis and hyalinization of the wall of blood vessel (arrow) H&E, 20 bar), N (The heart showing interstitial edema between cardiac muscle fiber (arrow) withcongestion of cardiac blood vessel (arrow head) H&E, 100 bar), O (The heart showing focal aggregation of heterphilis and mononuclear cells around cardiac blood vessels (arrow head)H&E, 20 bar), P (The lungs showing thickening of interalvealar septa by hemorrhage (arrow) and mononuclear cell infiltrations (arrow head)H&E, 20 bar), Q (The spleen showing necrosis of lymphocytes inside splenic tissue (arrow) with heterophilis infiltrations (arrow head) H&E, 20 bar), R (The intestine showing catarrhal enteritis represented by desquamation of superficial layer of intestinal villi (arrow head) with leukocytes infiltration in sub mucosa (arrow) H&E, 100 bar).



"Fig. 5", PCR amplification for UTR gene of DVH showing band size of ~250bp, M: Molecular marker,+CTL: Positive DVH strain, Lane S: Positive DVH samples.

DISCUSSION

Duck virus hepatitis was first described in 1950 in long island New York, causing severe losses in ducklings. The disease is an acute, highly contagious disease of young ducklings aged from 2 days to 3 weeks. Age resistance to disease is essentially complete from 7 weeks of age.[4]

Different strains of DVH cause disease ranging from mild to severe lesions in young ducks. The severe form is life threatening and causes highly economic losses to the industry of duck breeding but the previous studies of DVH infection have focused only on the pathogenicity of virus.[15]

In Egypt the causative agent of DVH is unknown but may be due to the used vaccine not produce good protection and it can be diagnosed by increased rate of

mortality in ducklings less than 3 week old and rapid occurrence of clinical signs.[16]

The most observed clinical signs of DVH were depression, eye closing, watery diarrhea and the affected bird fall on their side and kick spasmodically with head stretched upward and backward (opsithotonos position) and the mortality rate reached up to 80 % similar results were completely agreed with.[7] Macroscopically, the liver was enlarged, yellowish brown in color with hemorrhagic spots on its surface. The kidneys, spleen and lungs were enlarged and congested. The brain showed congestion in meninges with hemorrhagic patches beside congestion of intestinal mesentery was observed. Similar results were reported by.[8]

The target organ of DVH infection is liver which resulting in dysfunction of liver.[17] Microscopically, Hemorrhage, congestion, cell swelling, necrosis with infiltration of heterophiles and lymphocytes were observed in liver, kidneys, spleen, lungs, intestine, heart, gizzard, proventriculus, pancreas, brain and bursa. Our findings were partially agreed with.[10&11] The hepatic necrosis resulting in liver injury plays an important role in disease pathogenesis.[18]

Apoptosis can be induced by immune system to make clearance of virus while viruses developed strategies to prevent apoptosis of cells through proliferation of virus at first hours of infection.[19]

The differential diagnosis of DVH from other diseases such as avian influenza, salmonellosis and aflatoxicosis depended on rapid spread and sudden onset beside the more pathognomonic lesion which is hemorrhagic lesions in liver of duckling.[20]

Reverse transcriptase polymerase chain reaction (RT-PCR) was developed and is useful for confirming DVH infection.[21] RT-PCR technique considered rapid, specific, and simple detection of DHV-1 RNA which obtained from infected livers of ducklings.

The advantage of RT-PCR assay was for detection of DHAV RNA during outbreak before development of immune response. Also, the result of RT-PCR appears through 3-5 hours but other conventional laboratory detection methods as serological tests or virus isolation maybe take several days.[22]

The hygienic environments very important to prevent DVH due to the contagious nature of the disease beside injection of live attenuated vaccine can be used in prevention.

CONCLUSION

Duck viral hepatitis is highly infectious contagious diseases which cause high mortality and morbidity in young duckling so it causes highly economic losses in duck industry. The histopathology and PCR technique were the most accurate method for diagnosis of DVH.

Conflict of interest

The authors declare no conflict of interest.

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