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HISTOPATHOLOGY STUDY OF SEASONAL INFLUENZA VIRUS SUBTYPE (H1N1, H3N2) AND TYPE B ISOLATED FROM NASAL SWAB OF HUMANS IN ALNAJAF PROVINCE (IRAQ) AFTER THE PREPARATION OF A LOCAL INACTIVATED SUBUNITE AND WHOLE VACCINE

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Abstract- The present study included the section histopathology for rats (male) organs including (lung, trachea, kidney, spleen, heart) of a local inactivated subunit and whole vaccine appeared of all of both locally vaccine after seven months of vaccination and control histopathology unchanged compared with infected group after first month.

Keywords- Haematoxylin, Eosin

Introduction

Since 1918, influenza virus has been one of the major causes of morbidity and mortality, especially among young children. Though the commonly circulating strain of the virus is not virulent enough to cause mortality, the ability of the virus genome to mutate at a very high rate may lead to the emergence of a highly virulent strain that may become the cause of the next pandemic. Apart from the influenza virus strain circulating in humans (H1N1 and H3N2), the avian influenza H5N1 H7 and H9 virus strains have also been reported to have caused human infections, H5N1 H7and H9 have proven their ability to cross the species barrier for birds to humans and further replicate in humans [1,2].

Histopathological changes in various organs are similar, irrespective of the animal species and dose of indomethacin, and include widespread congestion and haemorrhage, thrombi in arterioles and capillaries of the kidney, heart, lungs and splenic fat, With widespread glandular degeneration and necrosis [3,4].

The pathologic findings in these contrasting cases of novel influenza A (H1N1) infection are similar to those previously described for seasonal influenza. The main pathologic abnormality in fatal cases is diffuse alveolar damage, but it may be overshadowed by an acute bacterial bronchopneumonia [5].

The main histopathological findings in all the examined lungs in the four herds were moderate to intense infiltration of leukocytes in the lamina propria and epithelium of small bronchi and bronchioles and mild to prominent peribronchiolar, peribronchial, and perivascular lymphocyte infiltrates. The lesions were multifocal and showed a lobular distribution and were observed in the majority of the examined sections, but they were most prominent in the cranial and central parts of the lungs. Moderate to severe bronchiolar and bronchial lesions were also observed in areas with no gross lesions as well as in one lung from herd 3 with no gross pathological registrations [6,7].

Necrotic bronchial and bronchiolar epithelium and attenuation of the epithelial lining with flattening of the epithelial cells were seen. Varying amounts of granulocytes, sloughed epithelial cells, cell debris, and mucus were present in the lumina of many bronchioles and small bronchi [8].

Other findings included hepatic central lobular necrosis, edema and degeneration of myocytes in the heart and extensive acute tubular necrosis in the kidney have been observed [9,10]. Although the gastrointestinal, hepatic, renal, and hematologic manifestations could suggest wider tissue tropism, there was no evidence of viral replication in organs outside the respiratory tract [11].

Material and Methods

Tissue Processing

Tissue processing was concerned with the diffusion of various substances into and out of stabilizes porous tissues. The diffusion process results from the thermodynamic tendency of processing reagents to equalize concentrations inside and outside blocks of tissue.

Preparation of Tissue Sections

Procedure was conducted in this study [12], which included the following steps:

Dehydration: The section passed in the concentrations of progressive forms of ethanol (70, 80, 90, and 95,100%). For the period (1.5 - 2 hours) in each concentration in order to remove water.

Clearing: Samples were cleared with xylene, twice for a period of (1.5-2 hours) for each time in order to remove the clearing solution from the tissue.

Infiltration: The samples were infiltrated with melted paraffin wax (56-58ºC) by placing the samples twice (1.5-2) hours each time.

Embedding: Buried samples in a container with specific templates to molten paraffin wax and left to harden.

Sectioning: Tissues were sectioned into (2-4µm) thickness using a rotary microtom and fixed models on the slides using the adhesive (Meyers albumin) and then put the slide in the oven at a temperature of (56- 58ºC) for (20) minutes to remove excess wax.

Staining: Histological Staining- Haematoxylin & Eosin (H&E) The

routine stain is that of hematoxylin and eosion and the technique are proven in [Table-1].

Table 1- The steps used to stain slides with H&E

Step	Reagent	Time	Step	Reagent	Time
1	Xylene	5min	8	Running tap water	10 min
2	Xylene	5min	9	0.1% Acid Alcohol	1 min
3	Absolute Alcohol	2 min	10	Running tap water	3 min
4	Ethanol (90%)	2 min	11	2% Eosin	1 min
5	Ethanol (70%)	2 min	12	Ethanol (70%)	2 min
6	DW	2 min	13	Ethanol (90%)	2 min
7	Haematoxylin	3 min	14	Absolute Alcohol	2 min
15	Dry, Xylene and mounted in DPX				

Microscopic Examinations: The stained section on the slide was examined by using light microscope (Olympus, Japan). Histopathological changes were also reported by experienced histopathology.

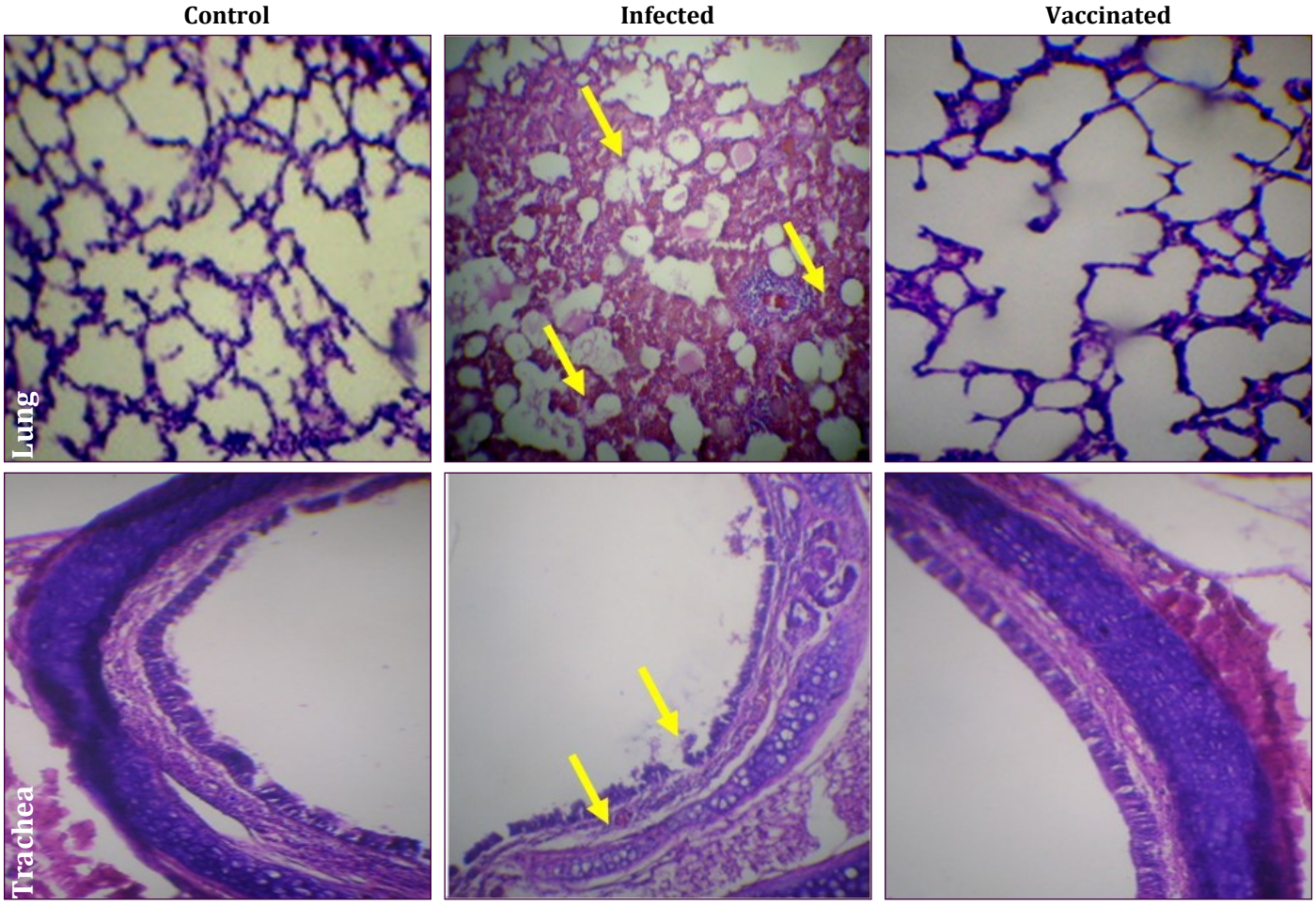
Result

Histopathological Changes

The microscopic studies proven varying degrees of cellular changes from mild to marked of infected group compared with vaccinated group.

Histological changes were observed in the lung showing necrotizing bronchiolitis. There is necrosis of the bronchiolar wall, with submucosal edema and vascular congestion. A mixed inflammatory cell infiltrate is present throughout the epithelial lining of the proliferation of the remaining epithelium. The lumen contain sloughed epithelial cells and mixed leukocytes. A large number of lymphocytes are seen infiltrating sub epithelial and peribronchiolar connective tissue. The alveolar air spaces contain edema fluid, thickening of the alveolar septa compared with the control lung, hemorrhage and hyperplasia smooth muscle in wall of pulmonary blood vessels, there is interstitial capillary congestion, and a peribronchiolar vessel strands of fibrin. There is fragmentation of surrounding bronchial muscle large togather with partial shedding of the mucosal lining and the appearance of cellular debris in destructed bronchiole.

Trachea showed epithelial disorganization including (mononuclear cell infiltration and intense nuclear hyperchromasis). Disruption of beam structure and dystrophy of hepatocytes in liver,, regions of necrobiosis, appearance of nacked nuclei and empty cytoplasm. Haemorrhages in glomerules and hyalinosis in renal tubules dystrophy of functional renal cell elements, tubular necrosis, glomerular destruction and formation of large lumens between the tubules, sever congestion. Devastation of cells in marginal zone of spleen, erythrocyte destruction in red pulpa with appearance of moderate haemorrhages. Heart indicated necrosis, hyalinization of muscle fibers with focal cellular infiltrations, damage and irregular arrangement and morphological change of myofibrils associated with increased interfibrillar distance infected experiential animal indicated cardiac muscle separation, myofiber loss, extensive subendocardial necrosis and inflammatory infiltrate into the myocardium and focal edema [Fig-1].



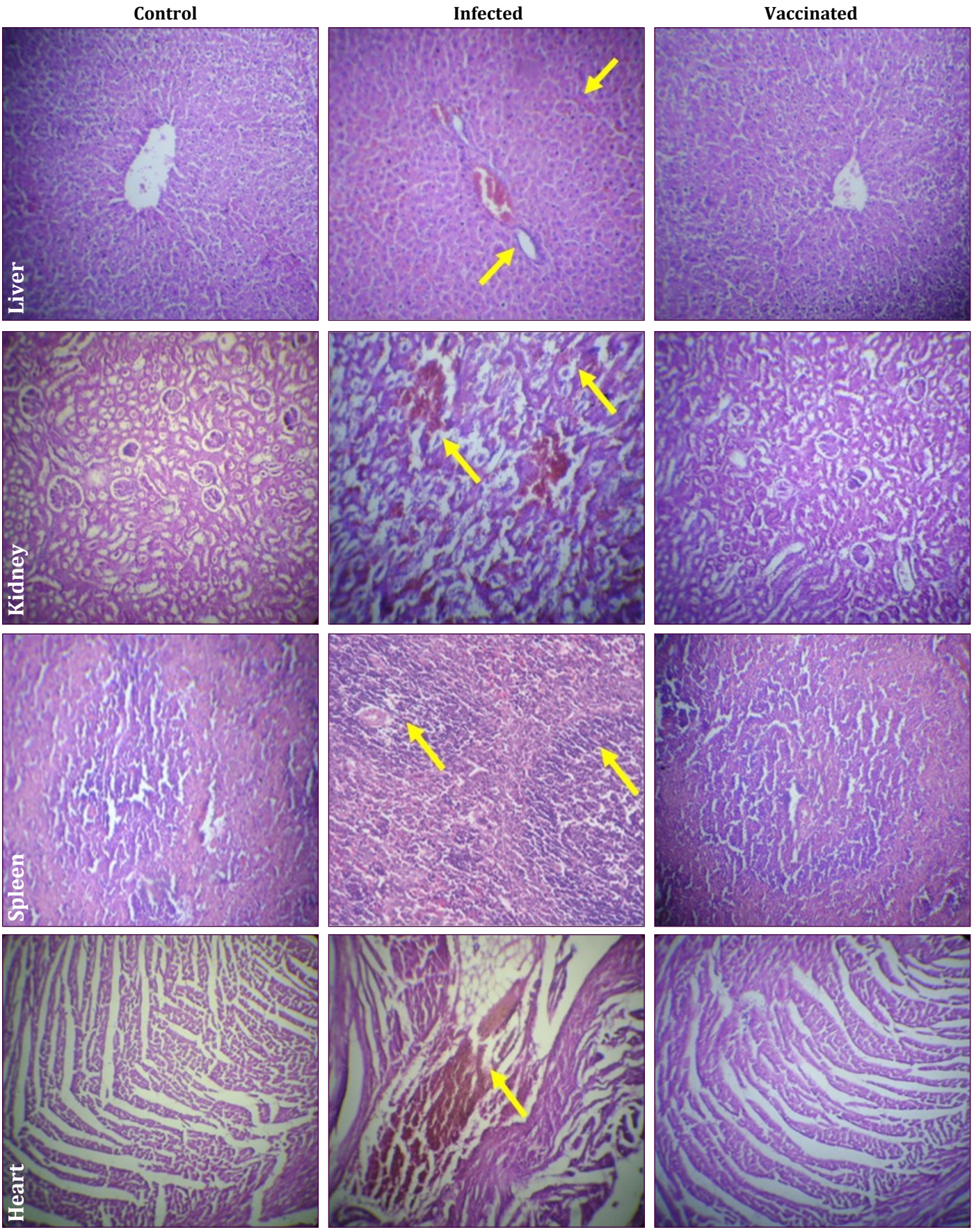


Fig. 1- Section from (Lung, Trachea, Liver, Kidney, Spleen, Heart) of vaccinated, infected and control groups indicated Histopathological of rat, Hematoxyline-Eosin stain, 40X.

Discussion

Histopathological Changes

There were obvious differences among histological sections of infected group of rats after infection compared with control and vaccinated group. After challenge test of vaccinated group with virulent virus there were no changes up to 4 months for animals given locally prepared inactivated vaccine (whole) and animals vaccinated with subunit vaccine. This observation is consistent with other studies [13,14].

Conflicts of Interest: None Declared.

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