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Pathological lesions of *Trichomonas gallinae* in Domastic pigeons (*Columba livia*) of Al-muthanna province, Iraq

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Abstract
This study was conducted to determine the incidence of Trichomoniasis in *Columba livia* , One hundred out of two hundred and fifty suspected pigeons were revealed clinical signs and lesion has been checked , the lesions included inflamed ulcerated white to yellow masses ulceration in the ulcerated oral cavities and/or gastrointestinal tracts of the affected pigeons. As well as showing a characteristic oscillatory movement of the parasite due to the movement of the front flagellum and the undulating membrane. Histological examination showed the presence of ulcers in the oral cavity, while in the tongue, histological examinations showed the presence of acanthus degeneration and severe infiltration of inflammatory cells, while in the gizzards, there was a separation between the skin of the stomach and the mucous membrane and atrophy of the simple tubular glands. In the liver, it showed hepatocyte dysfunction and hepatocyte necrosis in the focal region.
Keywords:pathological lesions, Domastic pigeons, Trichomonas gallinae

Introduction
Trichomonas gallinae, the etiology of avian trichomoniasis, is a flagellated, mitochondrial anaerobic protozoan belonging to the order Trichomonadida and the class Zoomastigophorea. This protozoa can be found in the upper digestive system of infected birds, a parasite that causes a disease that is parasitic across the world. Pathologic changes in clinically infected birds include mucosal inflammation and caseous lesions, which can clog the esophagus and ultimately result in starving death of the host (1) Birds infected with trichomonosis appears depressed, salivate excessively, emaciated, listless, ruffled and dull, have difficulty closing their mouth, display repeated swallowing movements with caseous, proliferative fibronecrotic lesions in the oropharynx and upper digestive tract which frequently lead to the death of the infected bird bystarvation. The high prevalence of *T. gallinae* infection and the low rate of pathological changes in pigeons were the main results of host-parasite relationship (2). Pigeons however are more susceptible to secondary organ invasion liver air sacs, lungs, and brain by virulent strains of the parasite which causes ulcers in the upper digestive tract which allow to enter the circulatory system, then access the liver where they causes lesions leading to high mortality. Lesions, particularly in the respiratory tract and esophagus, were created as a result of binary fission caused by Trichomonas gallinae. It can spread horizontally at common water and food sources and vertically in columbiformes through pigeon crop milk, which is supplied to young nestlings, despite the fact that the trichomonad has no intermediary host (3). Pigeon canker, which primarily the crop and the esophagus can affects, adding to the liver, and the lung can involved, is caused by *T. gallinae*. Cankers are made up of blood cells (mainly leucocytes),

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tissue remnants, parasitic degenerates, and other substances. They appear to represent the host's reaction to the presence of trichomonads. Columbidae are regarded as the T. gallinae's primary host , specially the domestic pigeons, which have long been thought to be the source of the disease. parasite prevalence is widespread (4), Although favoring pigeons and doves, T. gallinae can infect a broad variety of bird families, including bustard (5), songbirds (6) The developing infectious disease trichomoniasis, which is characterized by necrotic ingluvitisa, was initially identified in British finches in 2005 (7). Trichomonasis can be diagnosed through clinical symptoms, a direct examination, or a culture. Excessive salivation, prominent swallowing motions, and caseous diphtheritic membranes in the crop, mouth, and pharynx are some of the clinical indications of trichomoniasis. Trichomonasis is clearly suggested by distinctive nodules yellowis-white in color the crop, esophagus, and oral cavity. By identifying the pathogen during a microscopic inspection of the cheesy material, green fluids, and/or lesion, the infection is proven. The flagellate may be recognized with the help of a Giemsa stain

Materials and Methods

Samples collection and preparation :The most crucial stage in the diagnosis of *Trichomonas gallinae* infection is sample collection. *Columba livia* domestica pigeons One hundred out of two hundred and fifty suspected pigeons were revealed clinical Signs and lesion has been checked were acquired from a number of sources, including the local markets and Pigeons towers in Al-Muthanna Governorate. Birds' oral cavities were sampled.the preparation of all sample were done in the College of Veterinary Medicine/Poultry Diseases Laboratory at the University of Qadisiyah.the swabs were mixed with Phosphate Buffered Saline (PBS) using sterile cotton-tipped applicators for *T gallinae* examined under a light microscope at 40xby wet mount as reported by (8). **Preparation of swabs**

A thin rubber tube attached to a syringe was used to pull wet cotton swabs from inside the crop and from the pit after a modest amount of the physiological saline solution (normal saline solution) had been placed there. Then the slide is stabilized by flame and then stained with (Geimsa stain).then a clean glass slide should be examined undera light microscope and covered with another slide for a direct examination to further study the parasite.

Cultivation method

The positive sample of *T. gallinae* was cultivated in four media namely modified Diamond media (TYM) (Trypticase Yeast Extract media), with 10% inactivated foetal bovine serum, antibiotics (100 µg/mL ceftriaxone and 50 µg/mL ciprofloxacin) and fungicides (2.5 µg/mL amphotericin B) for three days at 37◦c. After 72 hours of growth and reproduction, the culture media were examined under a microscope.

Histopathology

To find any gross alteration in the internal organs, the pigeons were slaughtered, and the macroscopic appearances were noted. Internal organ samples, including those from the tongue, gizzard, and liver, were collected,then fixed with 10% formaldehyde solution, these tissues were processed by ethyl alchohol and cleard by xylene then embedded with paraffin wax using the histokinette (SLEE medical; Germany). Making aparafin blocke which cut into rotary microtome sections, and stained with hematoxylin and eosin stain (9).

Ethical approval

The study protocol was approved by the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

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Results

At post mortem, the macroscopic lesions are noticed which by characterized presence of white to yellow caseous masses ,ulcerated oral cavities and/or digestive tracts in infected pigeons, Detection of *T.gallinae* in the domestic pigeons swabs. The direct swab showed movement of parasite characteristic vibratory due to movement of anterior flagellae and the undulating membrane and showed a pear shape if it was the anterior part is wider than the posterior as in figure (1).

Histopathology

Examination of internal organs in the sacrificed pigeons (oral cavity, tongue, gizzard and liver) showed severe microscopic changes for example in the oral cavity there are suprabasal blister formation as in Fig. (2) and inflammatory cellular infiltration in the dermis as in Fig. (2) , (3). Also there is marked microscopic changes in the tongue, it characterized by formation of acantholysis (blue arrow) and severe infiltration of inflammatory cells as in Fig. (4).The gizzard showed detachment between gastric cuticle and mucosa and atrophy of simple tubular glands as in Fig. (5). Also there is loss of gastric cuticle, atrophy and necrosis of simple tubular glands, atrophy of beneath lamina propria of mucosa layer, and edema and necrosis of muscularis layer as in Fig. (6). The liver shows disarrangement of the hepatocytes, hydropic degeneration, and focal area hepatocytes necrosis are evident as in Fig. (7). Other sections of liver showed severe hydropic degeneration in which the hepatocytes swellen with prominent central nuclei and there are vacuoles in their cytoplasm as in fig. (8) and (9), also there is marked areas of necrosis in the hepatic tissue characterized by presence of nuclear changes in the hepatocytes like pyknosis, karyorrhexis and karyolysis of the nucleus as in fig. (9), infiltration of inflammatory cells mainly eosinophil also seen in the hepatic tissues as in fig. (10) and (11).

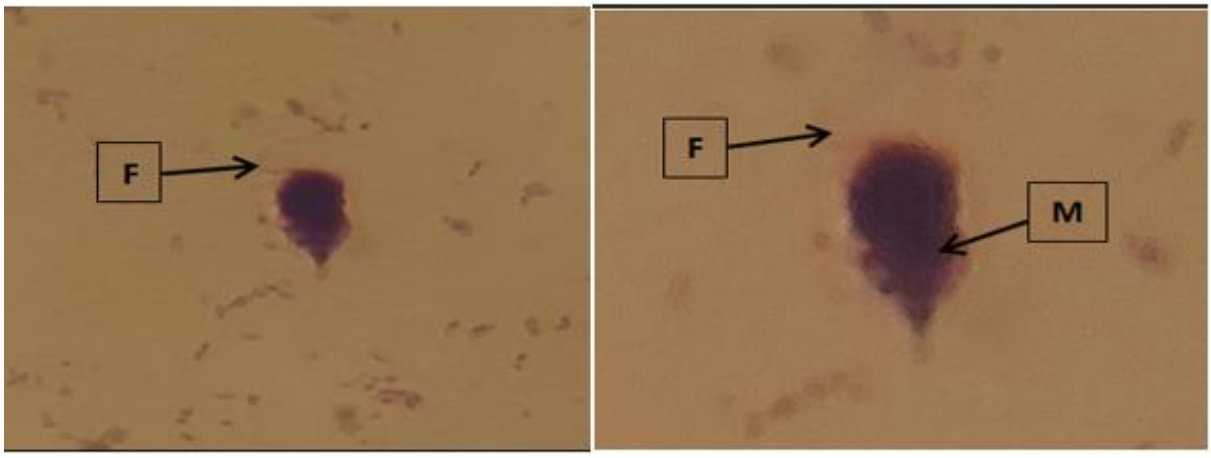

Figure (1): *Trichomonas gallinae* isolated from domestic pigeons with its anterior flagellae (F) and undulating membrane (M).

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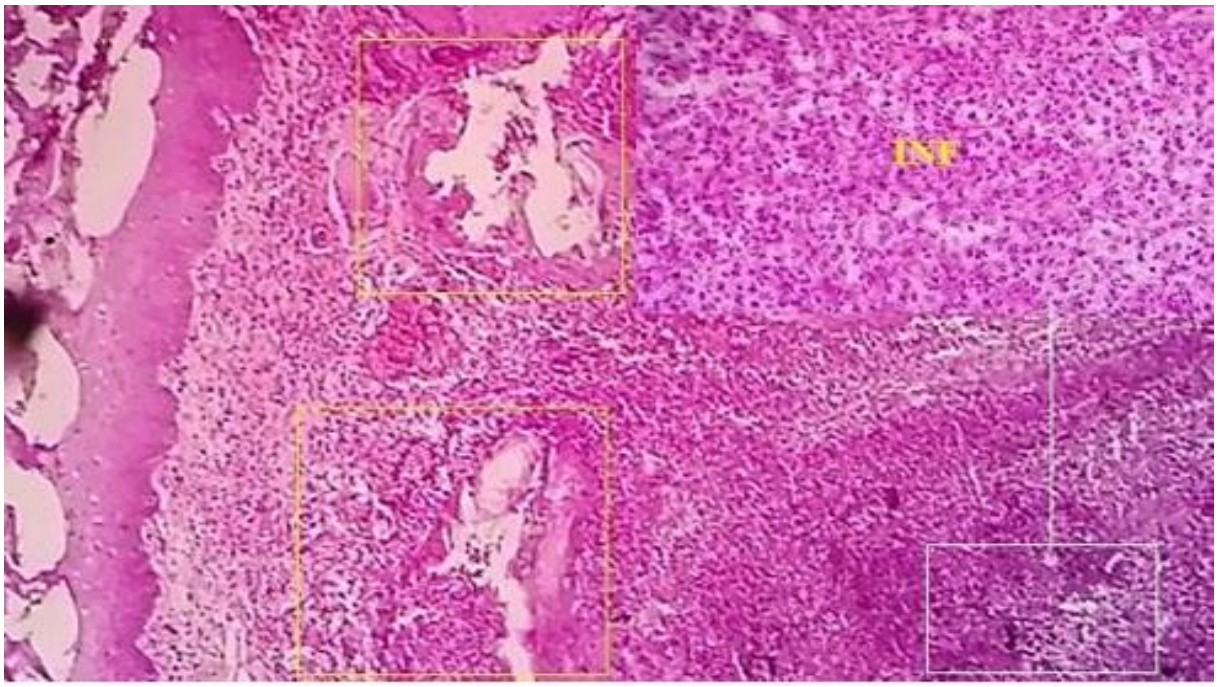

Figure (2): Micrograph of oral cavity of pigeon shows mucosal tissues suprabasal blister formation (yellow box) and inflammatory cellular infiltration in the dermis (white box). H&E, 100X. The focused area shows the severe infiltration of inflammatory cells(INF). H&E, 400X.

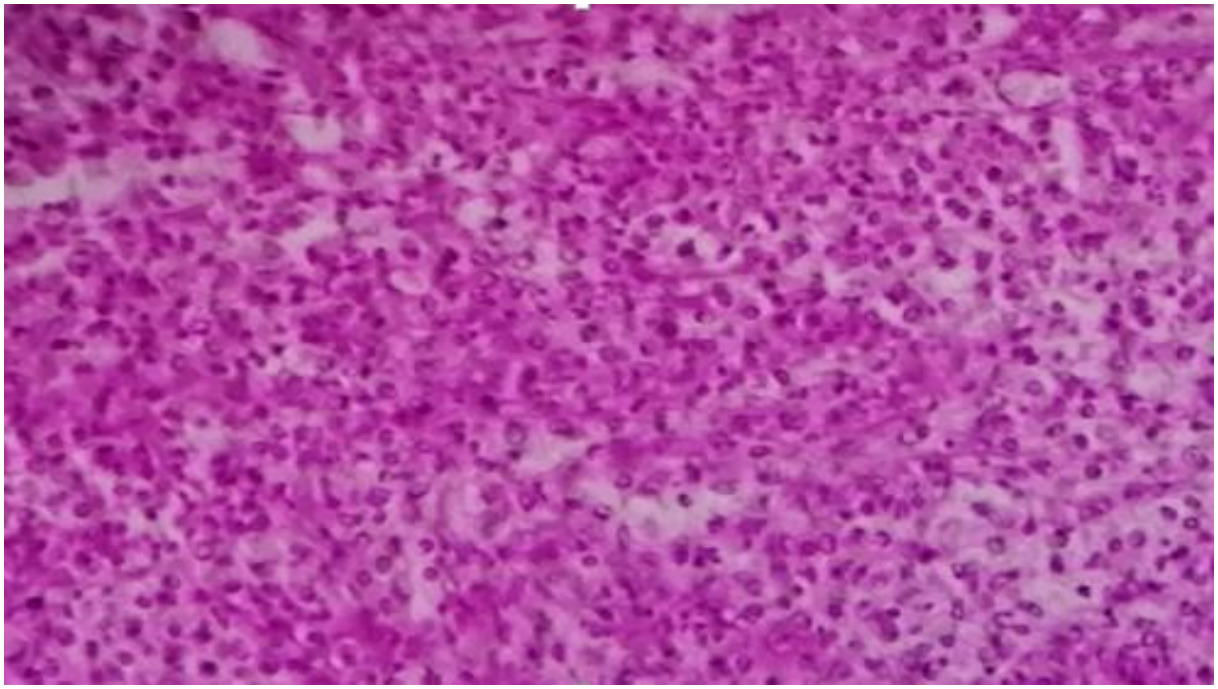

Figure (3): Micrograph of oral cavity of pigeon shows mucosal tissues shows severe infiltration of inflammatory cells in the dermis layer mainly macrophages. H&E, 400X.

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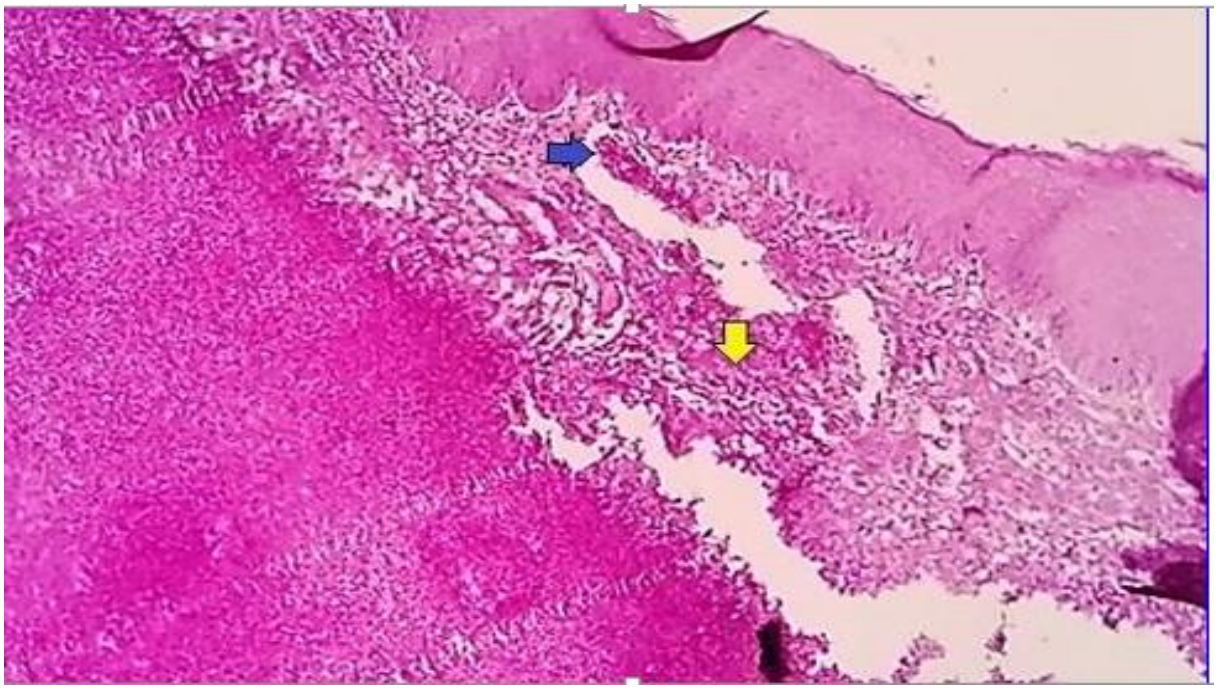

Figure (4): Micrograph of tongue of pigeon shows acantholysis (blue arrow) and severe infiltration of inflammatory cells (yellow arrow). H&E, 100X.

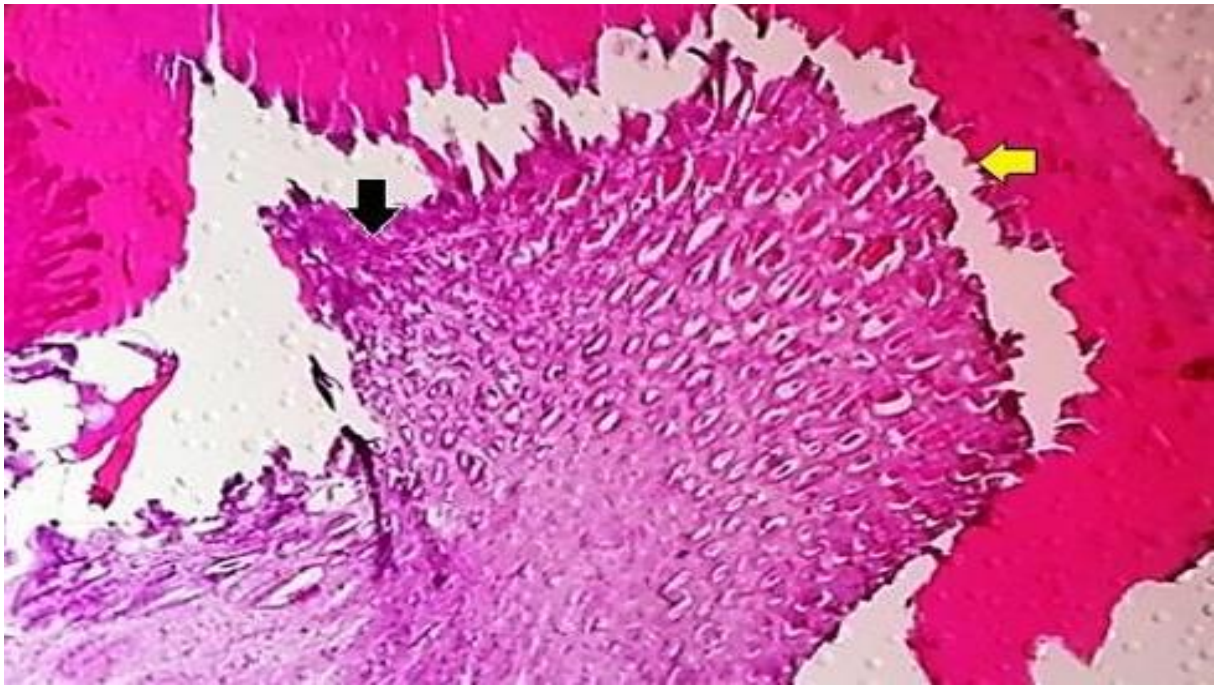

Figure (5): Micrograph of gizzard of pigeon shows detachment between gastric cuticle and mucosa (yellow arrow), and atrophy of simple tubular glands (black arrow). H&E, 100X.

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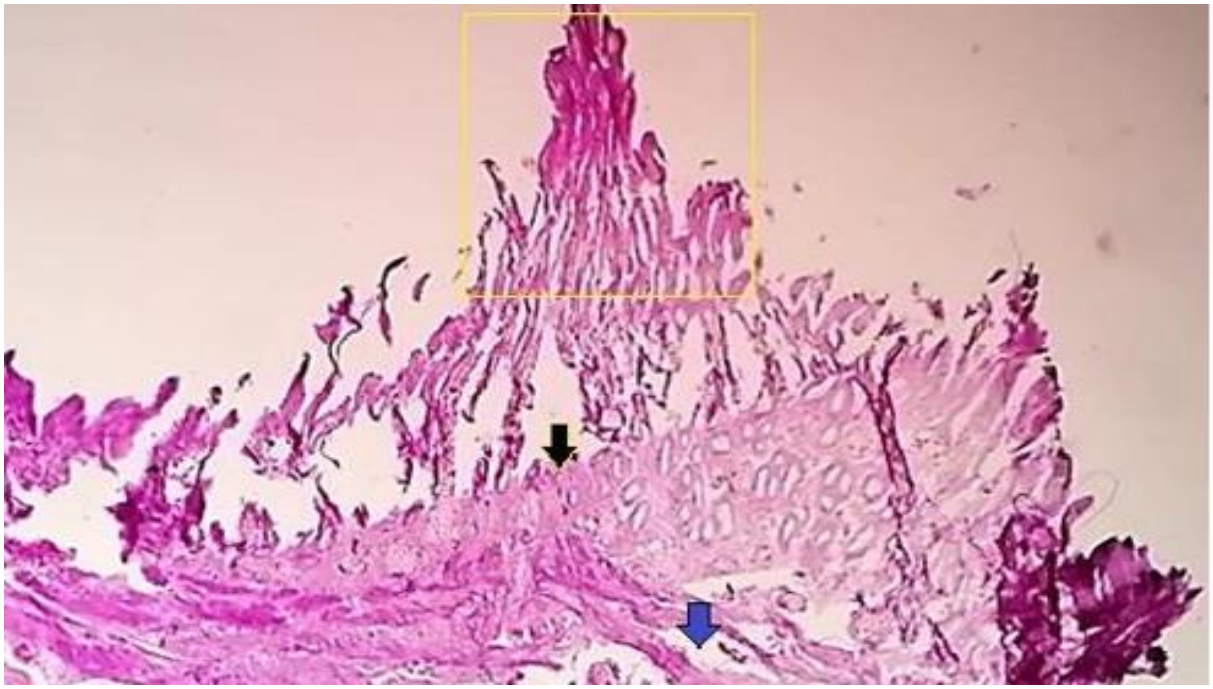

Figure (6): Micrograph of gizzard of pigeon shows loss of gastric cuticle, atrophy and necrosis of simple tubular glands (yellow box), atrophy of beneath lamina propria of mucosa layer, and edema and necrosis of muscularis layer. H&E, 100X.

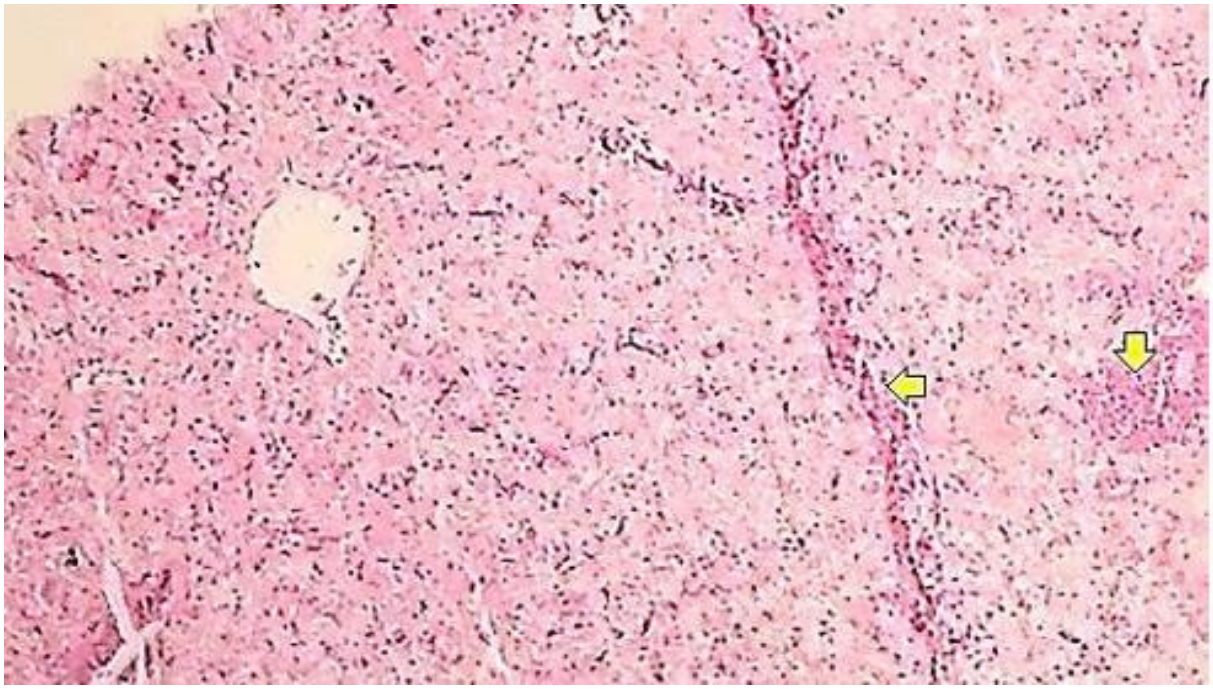

Figure (7): Micrograph of liver of pigeon shows disarrangement of the hepatocytes, hydropic degeneration, and focal area of necrosis (yellow arrows) of hepatocytes are evident. H&E, 100X.

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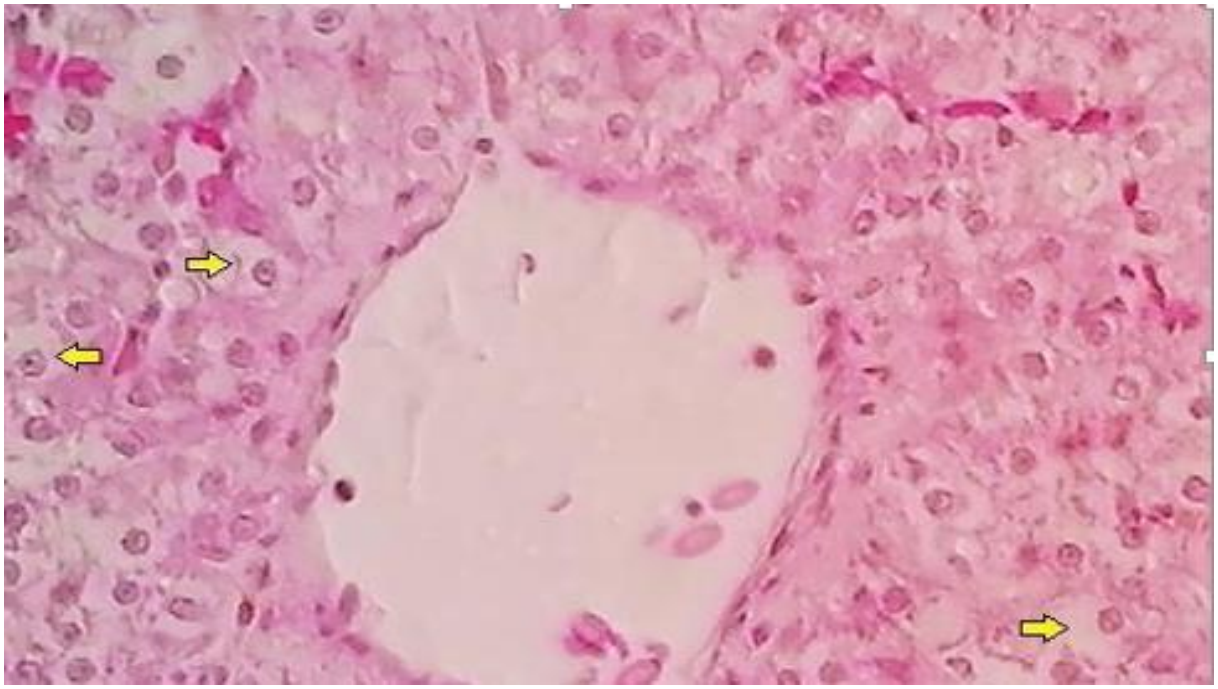

Figure (8): Micrograph of liver of pigeon shows hydropic degeneration of hepatocytes (yellow arrows). H&E, 400X.

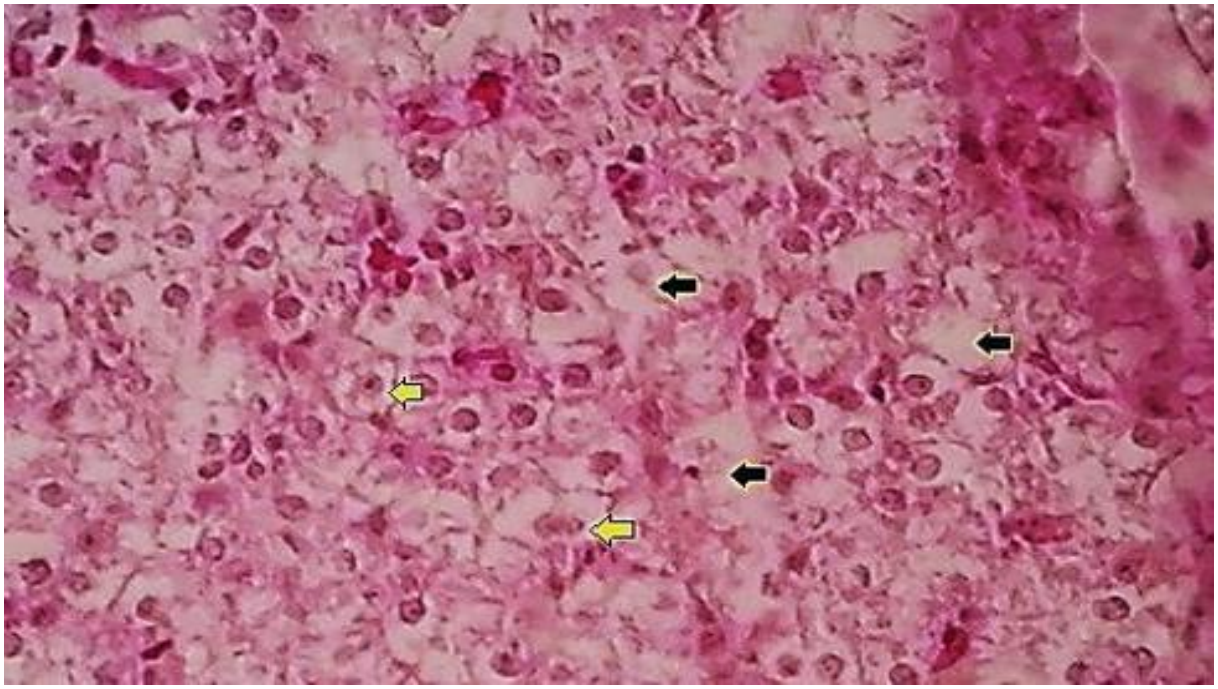

Figure (9): Micrograph of liver of pigeon shows hydropic degeneration (yellow arrows), and necrosis (black arrows) of hepatocytes. The pyknosis, karyorrhexis and karyolysis of the nucleus are evident. H&E, 400X.

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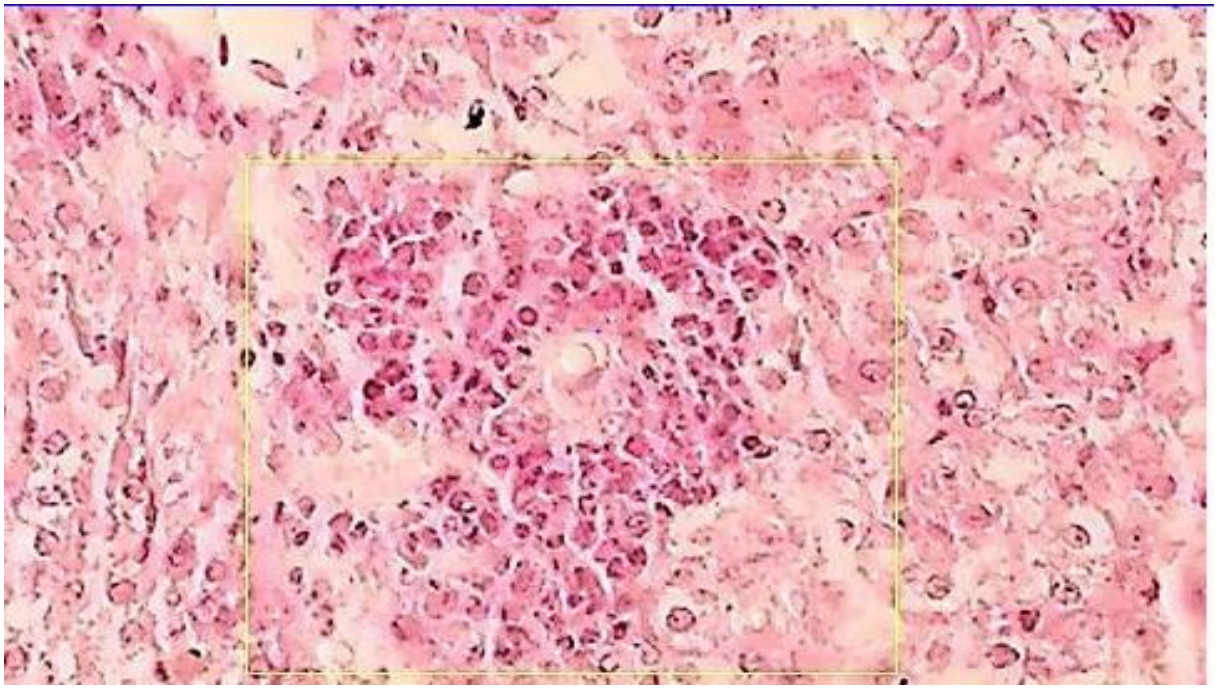

Figure (10): Micrograph of liver of pigeon shows hydropic degeneration andnecrosisofhepatocytes, and infiltration of inflammatory cells mainly eosinophils (yellow box) which interact locally with hepatocytes. H&E, 400X.

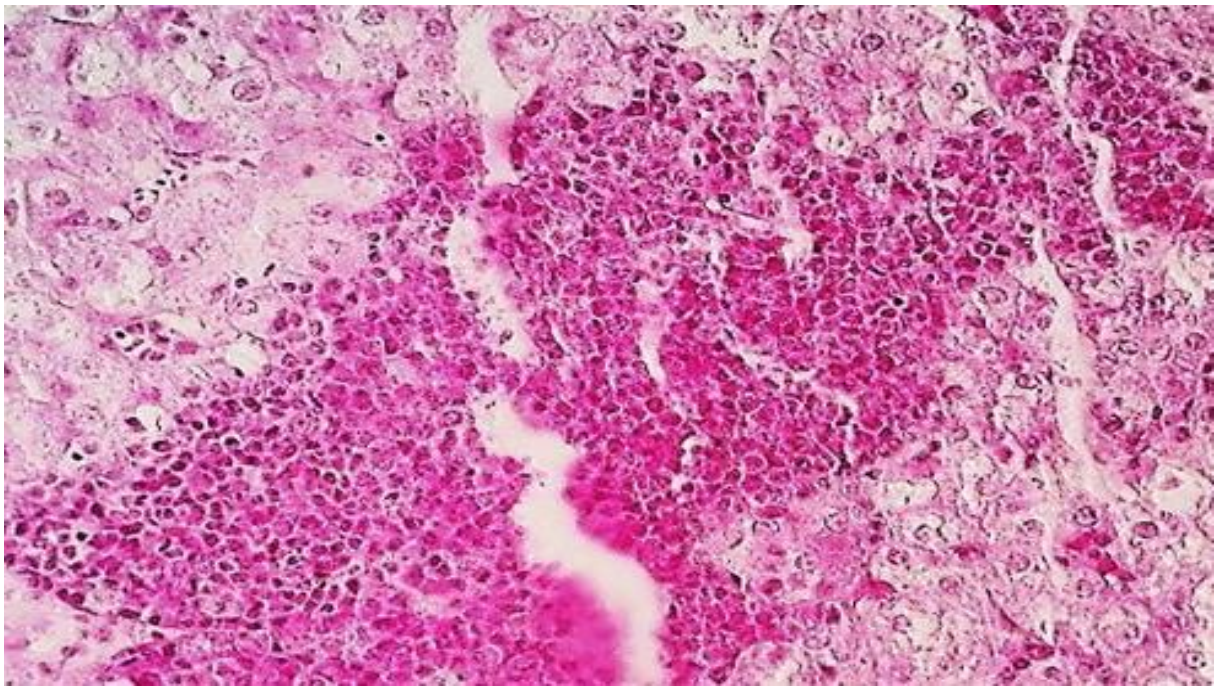

Figure (11): Micrograph of liver of pigeon shows hydropic degenerationand necrosis of hepatocytes,nd severe infiltration of inflammatory cells which interact locallywith hepatocytes. H&E, 400X.

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Discussion

The results of postmortem including white to yellow caseous masses inflamed ulcerated oral cavities and/or digestive tracts in infected pigeons. These results agreement with (10) and (11) who findings pathognomonic white-yellow caseous masses in mouth and trachea.The results of histological examination showed severe microscopic changes for example in the oral cavity there are suprabasal blister formation and inflammatory cellular infiltration in the dermis. Also there is marked microscopic changes in the tongue, it characterized by formation of acantholysis (blue arrow) and severe infiltration of inflammatory cells. While the study of (12) revealed the histological appearance in oral , esophagus and crop included irreversible tissue necrosis, congestion or decrease in blood flow, cells with a defensive reaction as a response to injury in the form of a vascular reaction to infection (inflammatory cells) and partial loss mucosa (erosion) and infiltration of inflammatory cells in the sub-mucosal layer. The result of the gizzard showed detachment between gastric cuticle and mucosa and atrophy of simple tubular glands. Also there is loss of gastric cuticle, atrophy and necrosis of simple tubular glands, atrophy of beneath lamina propria of mucosa layer, and edema and necrosis of muscularis layer. The study of (13) Revealed the histological appearance in gizzard showed separation of mucosal layer from muscular layer and separation of muscle. In the liver there is Sinusoidal congestion and kupffer cell hyperplasia also observed in the liver. Also there is disarrangement of the hepatocytes, hydropic degeneration, and focal area hepatocytes necrosis are evident as in Fig. (7). Other sections of liver showed severe hydropic degeneration in which the hepatocytes swellen with prominent central nuclei and there are vacuoles in their cytoplasm as in fig. (8) and (9), also there is marked areas of necrosis in the hepatic tissue characterized by presence of nuclear changes in the hepatocytes like pyknosis, karyorrhexis and karyolysis of the nucleus, infiltration of inflammatory cells mainly eosinophil also seen in the hepatic tissues (10) and (11). The above results agreed with studies of (14) showed multiple MNCs and heterophils aggregation necrosis in parenchyma, congestion and dilation in hepatic sinusoids, granuloma composed of MNCs and heterophils in liver tissue. Also, agreement with (11) who revealed the eosinophilic bodies with severe inflammatory cell infiltration .

Conflict of interest

The work here is with no conflict of interest.

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