The Third International Conference for Postgraduate Scientific Research, December 15, 2020
College of Veterinary Medicine, University of Baghdad

THE IRAQI JOURNAL OF
VETERINARY MEDICINE

eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Trichomonas gallinae Identification and Histopathological Study in Pigeon (*Columba livia domestica*) in Baghdad City, Iraq

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Received: 23 June 2020
Accepted: 1 September 2020
Published: 28 December 2020

DOI:
https://doi.org/10.30539/ijvm.v44i(E0).1022

Cite:
Fadhil LT, Faraj AA, AL-Amery AM. *Trichomonas gallinae* identification and histopathological study in pigeon (*Columba livia domestica*) in Baghdad city, Iraq. Iraqi J. Vet. Med. 28 Dec. 2020; 44(E0): 57-63.

A B S T R A C T

Trichomonas gallinae causes avian trichomoniasis, which is one of the most common protozoan infections in birds worldwide. Therefore, this study was conducted to investigate and identify the *Trichomonas gallinae* in domestic pigeons (*Columba livia domestica*) by microscopic examination (direct smear and Giemsa stain) and histopathological examination in Baghdad city, Iraq, during the period from beginning of October 2018 to end of March 2019. Giemsa-stained cytoplasm with light purple and nucleus with dark purple, clarification of flagella, nucleus, and cytoplasm very well. Histopathological findings of infected birds showed gross existence of yellowish white caseous necrotic material in the oral cavity and esophagus. The histopathological examination in the larynx, esophagus, trachea, crop, liver, and lung as infiltration of inflammatory cell mainly (heterophils); thickening of mucosa because of extensive infiltration of heterophils and disruption of esophageal gland; the thickness in bronchi wall of lung due to glandular hyperplasia and muscular fibroplasia, in liver focal necrosis of parenchyma with mononuclear cell (MNCs) infiltration and granuloma composed of MNCs and heterophils. The current study may contribute to determining the histopathological changes of esophagus, trachea, crop, and liver of trichomoniasis- infected pigeons.

Keywords: rabbits, Trichomoniasis, avian disease, histopathological changes, *Columba livia domestica*

INTRODUCTION

Birds form part of the human diet consumption. Some are used for breeding, decorative or entertainment purposes, while other birds are used for scientific experiments or teaching the art of mummification (1). However, birds like any animals can suffer from diverse types of disease (viruses or infectious diseases). Pigeons, for example, may infect with a group of external parasites such as lice and ticks (2). Pigeons can be infected by nematodes and cestoda parasites, such as the species of *Raillietina, Capillaria* and *Ascaridia*, as well as protozoa parasites such as *Eimeria* spp., *Cryptosporidium* spp. and *Trichomonas* spp., that leads to trichomoniasis (3). Avian trichomoniasis is protozoan disease caused by *Trichomonas gallinae*, which is a mitochondrial anaerobic protozoan, a flagellated parasite belongs to the class Zoomastigophorea and order Trichomonadida (4). *Trichomonas* infects the upper digestive tract of birds and this parasite infects a widespread of birds, such as pigeons, chickens, turkeys, and

other poultry worldwide (5). Infection by *Trichomonas gallinae* (*T. gallinae*) may occur without signs while it may lead to death with intermediate symptoms including anorexia, vomiting, ruffled feathers, diarrhea, dysphagia, dyspnea, weight loss, and increased thirst (6, 7). Several reports indicated that many large events of avian mortality have been associated with Trichomoniasis (8, 9). The main host of *T. gallinae* is domestic pigeon (*Columba livia domestica*), this pigeon breeds a role in the spread of this disease. Young pigeons are frequently infected by these protozoa and can die from the infection, but adult birds' species may act as a carrier with no sign (10). Therefore, this study was designed to study the histopathological changes that occur in some organ tissue of pigeon that infected with *T. gallinae* in Baghdad, Iraq.

MATERIALS AND METHODS

Birds and Study Area

The procedures used in this study were reviewed and approved by the scientific committee at the University of Baghdad's College of Veterinary Medicine in compliance with animal welfare ethical standards.

A total of 180 pigeons (*Columba livia domestica*) of different in ages and sexes were brought from local markets at Baghdad city, Iraq during the period from the beginning of October 2018 to the end of March 2019.

Microscopic Examination

Oropharyngeal swab samples were taken from the mouths and crop of pigeons randomly by using sterile, pre-moistened, cotton-tipped applicators which was confirmed by microscopic examination (wet mount method). The Trichomonas were identified if they motile and flagellated protozoa were observed under light microscope (11).

Staining

Swabs were taken from the oral cavity and crop of pigeons randomly. Swabs were blended with phosphate buffered saline, fixed with methanol, and dried at room temperature, then stained with Giemsa stain prepared by adding 1 part of Giemsa stain solution to 9 part of buffer solution (pH 7.2) (12). The slides were examined under the light microscope with oil immersion lens (100×). The parasite was identified according to (13).

Histopathological Examination

Samples were taken from the oral cavity, esophagus, crop, liver, larynx, trachea, and lung of 30 birds and placed in 10% neutral buffered formalin before being sent to the pathology laboratory at the College of Veterinary Medicine, University of Baghdad for histopathological examination. Formalin fixed samples were sectioned at 5 µm thickness and stained with Hematoxylin and Eosin (14).

RESULTS

Gross Examination

The gross lesion in the infected birds showed to be white to yellowish nodule of varied sizes in the oropharyngeal cavity associated with inflammation and ulceration. The lesion extended sometimes into esophagus, crop and proventriculus blocking of the respiratory tract and led to the death of the birds (Figure1).

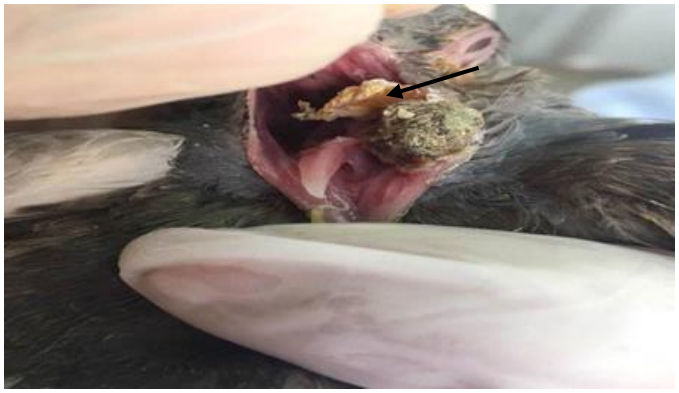

Figure1. Gross lesions of a domestic pigeon (*Columba livia domestica*) naturally infected with *Trichomonas gallinae* shows white material to yellowish in the pharynx and oral cavity of the upper gastrointestinal tract (black arrow)

Microscopic Examination

Microscopic examinations were done to the direct wet mount that taken from oral cavity, pharynx, and crop swab of the domestic pigeon. The motile organism was seen under the microscope using x40, showed vibrant active movement of the parasite was called jerky movement. The parasite was identified according to characteristic morphological features. It had a pear-shaped or oval shape with four anterior flagella and one undulating membrane in addition to having prominent axostyle at the posterior end of parasite (Figure 2).

Staining Examination

The primary purpose of staining was to optimize the visualization of key anatomic structures to facilitate accurate identification of an organism. In this study, it was used Giemsa stain. Microscopic structures of *T. gallinae* appeared when smears prepared and stained by Giemsa. Giemsa stained the cytoplasm with light purple and nucleus with dark purple color and visualizing the flagella and both of nucleus and cytoplasm were also very well (Figure 3).

Histopathological Examination

Larynx

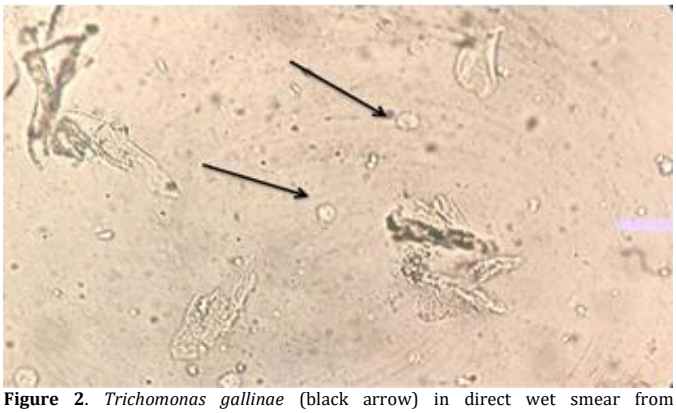

Figure 2. *Trichomonas gallinae* (black arrow) in direct wet smear from oropharyngeal of domestic pigeon (*Columba livia domestica*) (40×)

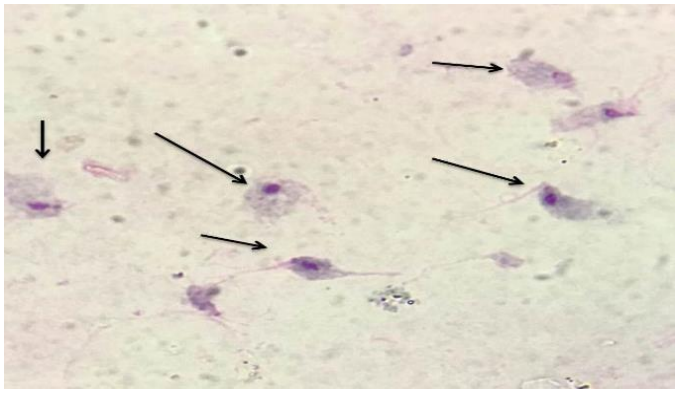

Figure 3. *Trichomonas gallinae* (black arrow) from oropharyngeal of pigeons (*Columba livia domestica*) stained with Giemsa stain (40×)

Multiple caseous necrotic lesions were recognized in lamina propria associated with focal epithelial sloughing, the laryngeal mucous glands showed various degree of sever degeneration with evidence of sloughed glandular epithelium with necrotic debris in lumen (Figure 4), the laryngeal was stratified epithelium exhibited focal keratinization with diffuse mononuclear cells (MNCS) infiltration and congestion in the lamina properia as well as slight lymphoid depletion recorded in sub mucosal layer. Cystic glandular dilation with flat epithelial lining and luminal debris were also noticed. In addition, laryngeal findings showed moderate to severe congestion of laryngeal muscular coated with evidence of vacuolation of muscle bundles, some laryngeal glands appeared lining with hyper plastic epithelium.

Trachea

The main histopathological finding of trachea was cystic dilation of major mucus glands with evidence of hyperactivity. This was associated with moderate hyperemia of tunica properia, severe degeneration of tracheal submucosal glandes with sub mucosal blood vessel congestion with no clear lesion in sub mucosal layer was noticed. Another section showed severe hyperatrophy of goblet cell with evidence of degeneration of some mucosal glands associated with vascular congestion of sub mucosal tissue (Figure 5).

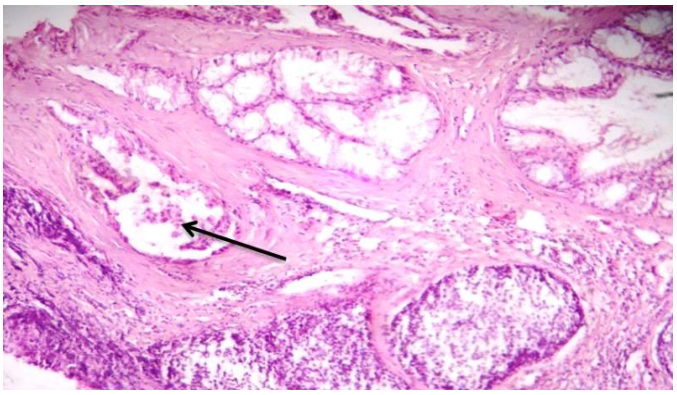

Figure 4. Histopathological section in larynx of domestic pigeon (*Columba livia domestica*) naturally infected with *Trichomonas gallinae* showed sloughed epithelum of larygeal gland with necrotic debris (black arrow) (H&E stain, 40×)).

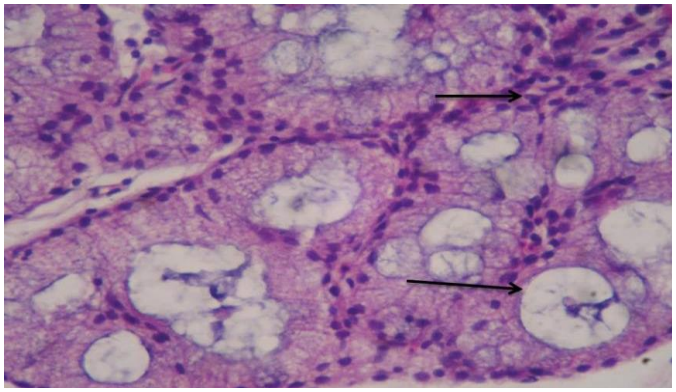

Figure 5. Histopathological section of trachea in domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows hyperactivity of sub mucosal glands with moderate MNCs infiltration (H&E stain, 40×)

Esophagus

The stratified squamous epithelial of esophagus in many parts was necrotized and debris was seen in the lumen. Moderate to severe disruption of esophageal submucosal glands with cellular infiltration and blood vessel congestion were also noticed in (Figure 6). There was focal MNCS infiltration composed mainly of lymphocyte that was seen between muscular bundles (Figure 7). Intense MNCS infiltration between mucus gland was also seen (Figure 8).

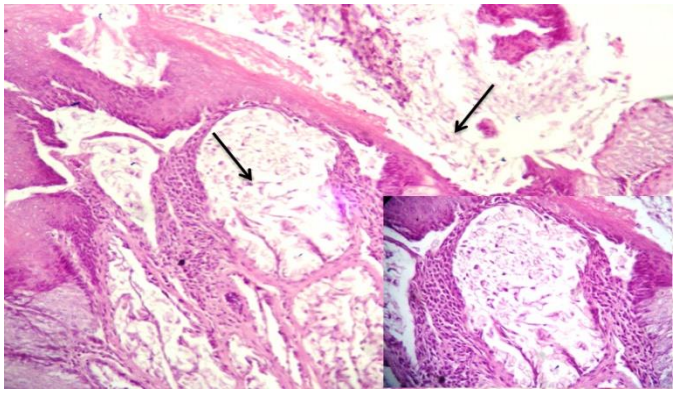

Figure 6. Histopathological section in esophagus of domestic pigeon (*Columba livia domestica*) naturally infected with *Trichomonas gallinae* shows moderate to severe disruption of esophageal glands with cellular infiltration (H&E stain, 40×)

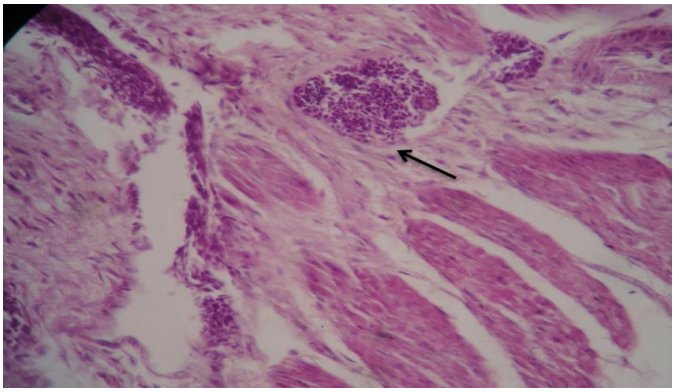

Figure 7. Histopathological section in esophagus of domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows focal MNCS infiltration composed mainly of lymphocyte that was seen between muscular bundles (black arrow) (H&E stain, 40×)

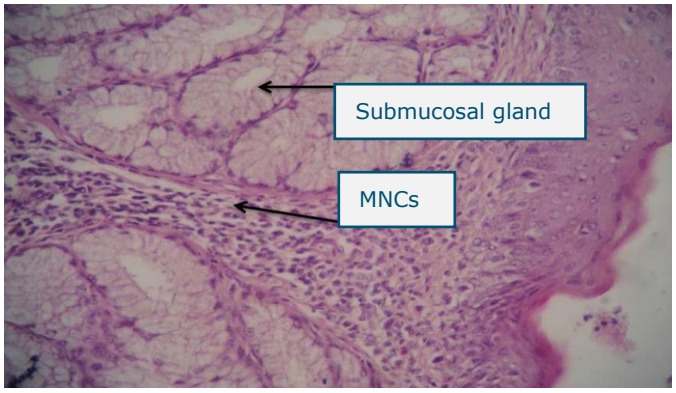

Figure 8. Histopathological section in esophagus of domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows intense MNCs infiltration between submucosal glands (H&E stain, 40×)

Crop

Crop lesion was characterized by focal surface mucosal desquamation with focal moderate necrotic lesion of the mucosal epithelia appeared with sloughed epithelium and focal MNCs with heterophils aggregation was also seen (Figure 9).

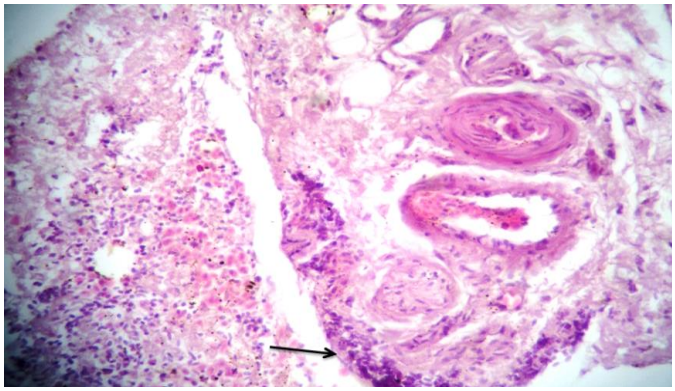

Figure 9. Histopathological section in crop of domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows focal MNCS with hetrophils aggregation (black arrow) (H&E stain, 40×)

Proventriculus

The mucosal fold of proventriculus showed moderate sloughing with mild atrophy of properial lymphoid tissue, while other section showed severe congestion of sub mucosal layer with diffuse MNCS infiltration in tunica properia. No clear lesion was detected in periventricular glandular tissue**,** while other observation revealed focal disruption of periventricular glandular tissue with diffuse sub mucosal MNCS infiltration (Figure 10).

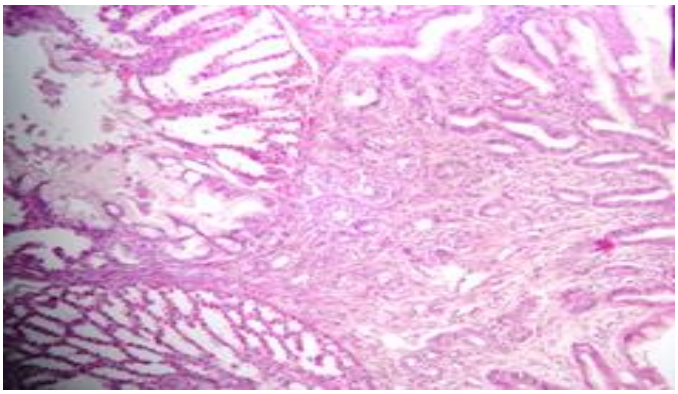

Figure 10. Histopathological section in proventiculas of domestic pigeon (*Columba livia domestica*) naturally infected with *Trichomonas gallinae* shows focal disruption of proventicular glandular tissue with diffuse sub mucosal MNCS infiltration (H&E stain, 40×)

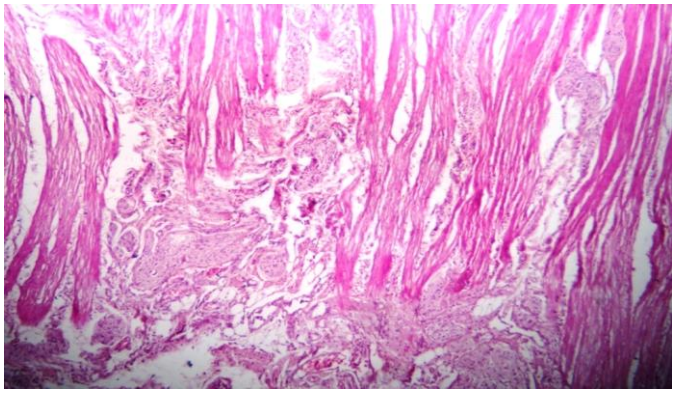

Figure 11. Histopathological section in proventriculus of domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows severe fragmentation of muscle bundles and separation with intra-muscular edema (black arrow) and blood vessel congestion (H&E stain, 40×)

Focal necrotic foci with congestion were observed in lamina propria accompanied with loss of sub mucosal papillae**,** as well as sub mucosal shortening of mucosal folds, together with severe fragmentation of muscle bundles and separation with intra-muscular edema and blood vessel congestion (Figure 11).

Lung

Pulmonary parenchyma showed multiple necrotic foci were surrounded by various type of MNCS forming granulomatous like lesion with giant cell formation (Figure 12) associated with severe destruction in adjacent parenchyma. The primary bronchi also showed moderate thickness of its wall due to glandular hyperplasia and muscular fibroplasia. Severe pulmonary congestion was detected mainly in arterial tissue (Figure13) while slight changes were detected in para bronchi as well as diffuse

MNCS infiltration with neutrophils result in severe consolidation of pulmonary tissue.

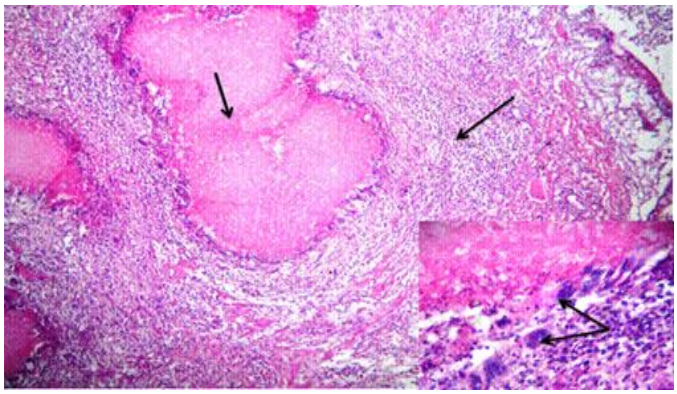

Figure 12. Histopathological section in lung of domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows multiple necrotic foci surrounded by various type of MNCS forming granulomatous like lesion with giant cell formation (black arrow)(H&E stain, 40×)

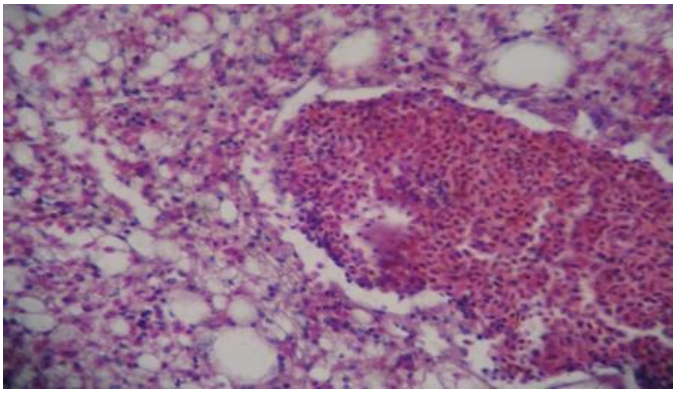

Figure13. Histopathological section in lung of domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows severe pulmonary congestion (black arrow) (H&E stain 40×).

Liver

Multiple MNCS and heterophils aggregation were observed in liver tissue mainly around blood vessels with evidence of sinusoidal dilation, while other finding showed granulomatous like lesion composed mainly of MNCS. Severe vacuolation of hepatocytes were also observed with evidence of hepatic steatosis, per vascular MNCS aggregation accompanied with appearance cytoplasmic fat droplets in adjacent hepatocytes, large MNCS aggregation was noticed in liver parenchyma with cellular swelling of adjacent hepatocytes. While other sections showed focal necrosis of parenchyma with MNCS and heterophils infiltration accompanied with individualization and atrophy of adjacent hepatocytes (Figure 14).

Portal MNCS infiltration with heterophils also recorded together with evidence of portal fibrosis and ductal dilation. Severe congestion and dilation of hepatic sinusoids resulted in disruption of hepatic cords (Figure 15) accompanied with severe congestion and dilation of central vein were also reported in many sections accompanied with focus MNCS infiltration, moderate portal enlargement due to marked MNCs infiltration and portal vein congestion, with evidence of portal fibrosis and focal perivascular infiltration**.** Other hepatic manifestations showed moderate number of apoptosis mainly adjacent to the central vein and granuloma was observed composed of MNCS and heterophils (Figure16). There was massive hemorrhage and sinusoidal congestion resulting in severe atrophy of hepatic cords.

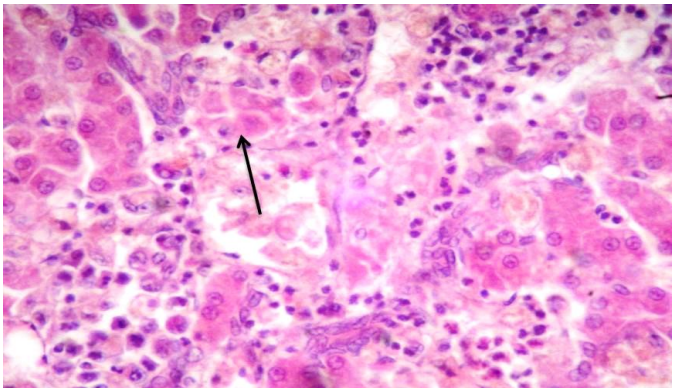

Figure 14. Histopathological section in liver of domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows focal necrosis with MNCS and hetrophils infiltration accompanied with individualization of adjacent hepatocytes (black arrow) (H&E stain, 40×)

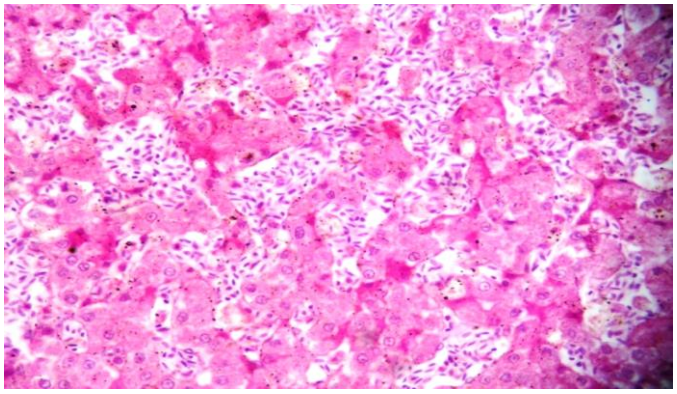

Figure15. Histopathological section in liver of domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows severe congestion and dilation of hepatic sinusoids result in disruption of hepatic cords (H&E stain, 40×)

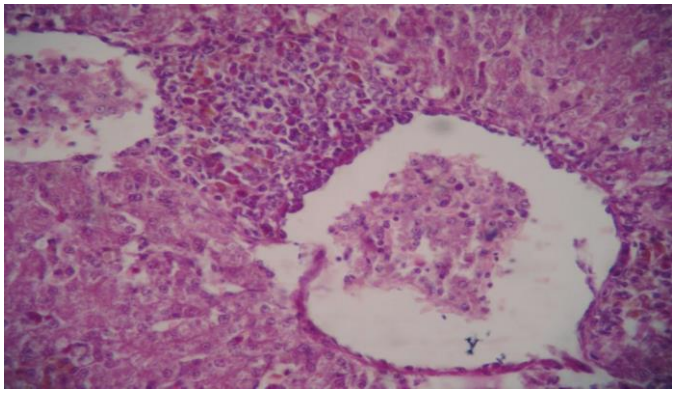

Figure16. Histopathological section in liver of domestic pigeon (*Columba livia domestica*) infected with *Trichomonas gallinae* shows granuloma composed of MNCs and hetrophils (H&E stain, 40×)

DISCUSSION

Occluded mouth and esophagus of pigeons with cheesy white to yellow-colored materials were also described previously by (14).

Histopathological examination of tissues in domestic pigeon that infected naturally with *T. gallinae* in the present study revealed multiple caseous necrosis were mainly seen in larynx, esophagus, and lung and diffuse MNCS infiltration and congestion in lamina propria. Severe degeneration in mucosal gland, cystic glandular dilation with luminal debris in the larynx, trachea revealed cystic dilation mucus glands hyperactivity with moderate hyperemia of tunica propria and severe degeneration of tracheal glandes with sub mucosal blood vessel congestion were similar to observation seen with El-khatam (14) in Egypt when it was recorded in trachea and larynx severe inflammatory cell infiltration. Abd El-Rahman (15) revealed existence of necrosis and infiltration of inflammatory cells mainly eosinophil in the mucosa of esophagus and these results agreed with our study that revealed necrosis of epithelial cell, focal MNCS infiltration especially lymphocyte between muscular bundles**,** infiltration of inflammatory cell between mucus glands. The crop and proventriculus revealed desquamation and sloughed in epithelial cell**,** focal MNCs with heterophils aggregation, infiltration of inflammatory cells mainly heterophils, necrosis in sub mucosa, focal disruption of periventricular glandular tissue with diffuse sub mucosal MNCS infiltration, edema, and congestion in the proventriculus. The above results were in agreement with the results of Abd El-Rahman et al. (15) in Egypt, Begum et al. (16) in Bangladesh, Al-Sadi and Hamodi (17) in Mosul, and Jaafar (18) in Baghdad in which they recorded necrosis in the sub mucosa of crop and desquamation in the epithelial cells of proventriculus.

lung showed multiple necrotic foci surrounded with various type of MNCS forming granulomatous like lesion with giant cell formation and congestion in pulmonary tissue, moderate thickness in bronchi wall due to glandular hyperplasia and muscular fibroplasia, and liver tissue showed multiple MNCs and heterophils aggregation necrosis in parenchyma, congestion and dilation in hepatic sinusoids, granuloma composed of MNCs and heterophils. The above results agreed with studies of (8, 14). The current study may contribute to determining the histopathological changes in the esophagus, trachea, crop, liver, and liver of pigeons that infected with trichomoniasis.

ACKNOWLEDGEMENTS

The authors are appreciated the faculty members of the College of Veterinary Medicine, University of Baghdad for providing all facilities for completing this work.

FUNDING

These authors declare that above-submitted work was not funded by any governmental or private funding source nor supported by any financial projects.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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داء المشعرات ودراسة التشريح المرضي في الحمام (*Columba livia domestica*) في مدينة بغداد، العراق.

ليلى طاهرفاضل، ازهارعلي فرج،عامر مرحم العامري

فرع الطفيليات، كلية الطب البيطري، جامعة بغداد، العراق

الخلاصة

داءالمشعرات هوواحد من أهم أنواع العدوى التي تصيب الطيورفي العالم والتي تسببها طفيلي *Trichomonas gallinae*. لذلك،أجريت هذه الدراسةلاستقصاء ومعرفة داءالمشعرات في الحمام المنزلي عن طريق الفحص المجهري (المسحة المباشرة وصبغة كيمزا) في مدينةبغداد،العراق،خلال الفترةمن بداية تشرين الاول 2018 إلى نهاية اذار 2019. أظهرت النتائج المرضيةللطيورالمصابة.بوجود كتل بيضاء- مصفرة اللون بشكل واضح في تجويف الفم والمريء.اظهرت استخدام صبغة الكيمزا بصبغ السيتوبلازم باللون الأرجواني الفاتح والنواة مع اللون الأرجواني الداكن ، وتوضيح الأسواط والنواة والسيتوبلازم بشكل جيدًا.اما الفحص النسجي للحنجرة ,المريء,القصبةالهوائية, الحوصلة, الكبد والرئة فاشارت بارتشاح الخلايا الالتهابية بشكل رئيسي(العدلات) ،كماهناك تثخن في الغشاء المخاطي للمريء بسبب تخلل واسع النطاق من البثرات.كان هناك سماكة في الغشاء المخاطي بسبب تسلل واسع النطاق من العدلات واختلال غدة المريء وتثخن في جدارالرئة والشعب الهوائية بسبب تضخم الغدة تحت المخاطية وتليف العضلي،تنخرفي الكبدبسبب ارتشاح الخلايا الوحيدة النواة، وتكون الورم الحبيبي الذي يتكون من العدلات والخلايا الوحيدة النواة. ساهمت الدراسة الحالية لتحديد التغيرات النسيجية المرضية في المريء والقصبة الهوائية والحوصلة وكبد الحمام المصابة داء المشعرات.

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الكلمات المفتاحية: داء المشعرات، مرض الطيور، التغيرات النسيجية، *Columba livia domestica*