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Diagnosis of *Histomonas meleagridis* in naturally infected turkeys in different areas of Tikrit and Hawija District, Iraq

Qudama Obaid¹, Omaima Mahmood²

^{1,2} Department of Microbiology, College of Veterinary Medicine, University of Tikrit, Iraq.

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Corresponding Author:

Name: Qudama Obaid

E-mail: qa230085pve@st.tu.edu.iq

Tel: :

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ABSTRACT

The current study included microscopic diagnosis of 25 samples of liver and cecum from naturally infected turkeys with *Histomonas meleagridis* by direct smear using giemsa stain .This study is considered the first in Salah al-Din and Kirkuk provinces / Iraq. The results of microscopic examination showed the presence of the parasite *H.meleagridis* in (22) liver samples (88%) and in (21) cecum samples (84%) where the parasite appeared as amoebic form in a spherical or oval shape and a size ranging (4_12 µm).

Clinical signs are Depression, loss of appetite, body weight loss with yellow foul smell diarrhea in some cases, as well as head drooping and ruffled feathers and the cyanosis head was observed only in a few cases of Poult. Macroscopic examination of cecum and liver samples showed an increase in thickness of cecum walls, in addition to the presence of bleeding and hard cheesy materials in the cecal cavity, while in the liver there was an enlargement and necrotic foci of different sizes and shapes.

1. Introduction

Histomonas meleagridis, anaerobic protozoan parasites , order Trichomonadida causes disease called Histomoniasis [1]. Common synonyms include (infectious typhlohepatitis, Histomonosis and blackhead disease) is an Infectious disease that infect gallinaceous birds, especially turkeys and chickens [2]. Cushman in (1893) described first known

outbreak that occurred in turkey flock in Rhode Island. Two years later Smith described the disease and attributed it to the protozoan *Amoeba meleagridis* obtained from liver lesions. After that , Tyzzer in (1920) renamed this protozoan *Histomonas meleagridis* [3].

The parasite appears in two forms the flagellated form that found in the cecal

cavity and anon- flagellated form when invades tissues .Also a cyst like stage may be seen under unfavorable conditions [4].

There are two ways to complete protozoan life cycle by direct contact from bird to bird in complete absence of caecal worm *Heterakis gallinarum* via “cloacal drinking” , or indirect by ingestion of embryonated eggs of the caecal worm *H. gallinarum* that contain *H. meleagridis* trophozoites or via ingestion of earth worm that eaten *H. gallinarum* eggs [5].

The pathogenesis start when the protozoan reach the cecal lumen of the host and invade the mucosa layers of the cecal wall and causes ulceration, inflammation, and that may lead to peritonitis , and when the parasite migrate to liver throw portal vein resulting in variable hepatic lesions [6]. Mortality may reach to 100% in turkeys . Ruffled feathers, drooped wings, depression and sulfur yellowish diarrhea are the most clinical signs companied with infection [7].

Diagnosis of disease was based on history of the disease , clinical signs , gross and histopathological lesions in liver and cecum and molecular diagnosis [8]. The disease is widely spread in many countries [9]. chemicals compounds like arsenicals or nitroheterocyclic are one of the drugs that used for control of the disease until the drugs were forbidden for the reasons of food safety ,Since that, the disease re- appeared in many parts of the world [10].

2. Material and methods

2.1 Sample collection

A total of 25 samples of naturally infected turkeys (male and female) of different ages were collected from different areas of Tikrit district and its

suburbs in Salah al-Din province and Hawija city in Kirkuk province in Iraq. The samples collected from the beginning of September 2023 until mid-January 2024 and was transferred from the place of collection to the College of Veterinary Medicine,parasite laboratory at Tikrit University.

2.2 Gross examination

Bird autopsy was done inside the laboratory under sterile conditions to preserve the samples from contamination . After that, liver and cecum samples were taken and a macroscopic examination was conducted on them to note the lesions that may appear, especially the lesions characteristic of the disease.

2.3 Microscopic examination

Direct smear were taken from the liver and cecum and stained with giemsa stain according to [11] .Then placed on a glass slide and left to dry through the air ,After drying, methanol solution was added for (10) minutes until it evaporates ,and stained with 5% giemsa stain diluted with tap water for 20 minutes, excess dye was then eliminated by washing the slide with tap water and left the slide to dry and examined by light microscope (Olympus,Philippines) under the power x100.

3. Result

3.1 Clinical observation

Clinical examination was conducted on turkey samples, where Most of the infected and diagnosed birds in this study suffer from depression , loss of appetite and body weight ,yellow diarrhea with a bad smell

for some cases, and we also noticed other signs such as head droop and ruffled feathers and the cyanosis head was observed only in a few cases at young ages.

3.2 Gross observation

Cecum and liver samples showed distinctive lesions of the disease. In cecum, we noticed the presence of solid cheesy materials inside the cecal lumen that appeared in some cases, in addition to an increase in the thickness of the cecum

walls and bleeding as shown in Figures (1). In Liver, there is an enlargement and discoloration to the dark color, in addition to the presence of lesions of different shapes and sizes, some of which appeared in the form of yellow nodes alone or coalescence as shown in Figure (2), and others appeared in the form of very few small white color nodules (pinpoint size) in separate areas of the liver surface and some others appeared in the form of pale white nodes resembling lymphoma cancer as shown in Figure (3).

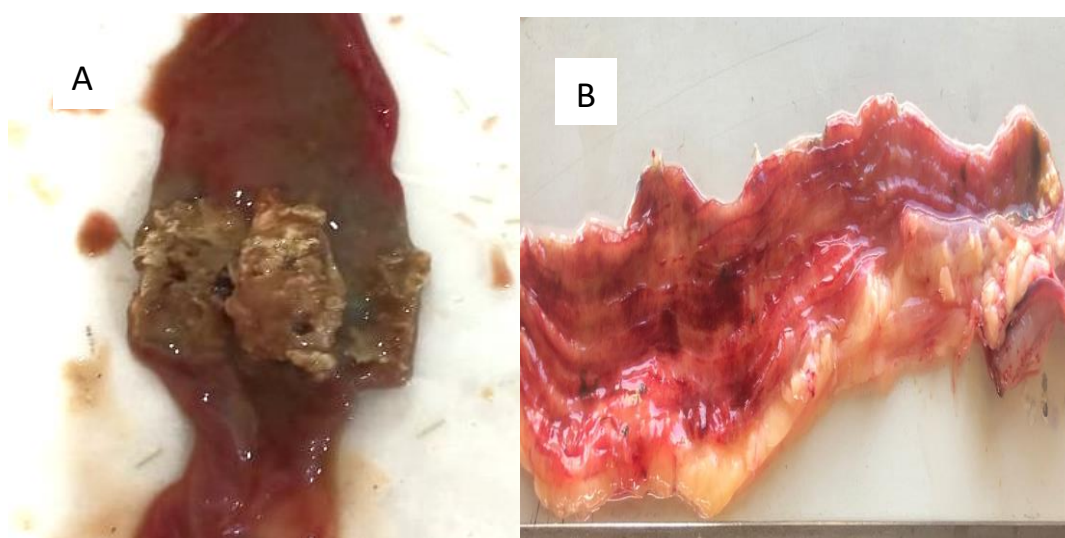


Figure (1): (A)_The presence of hard cheesy materials in the cecum. (B) An increase in the thickness of the wall of the cecum in addition to the presence of necrosis and bleeding.

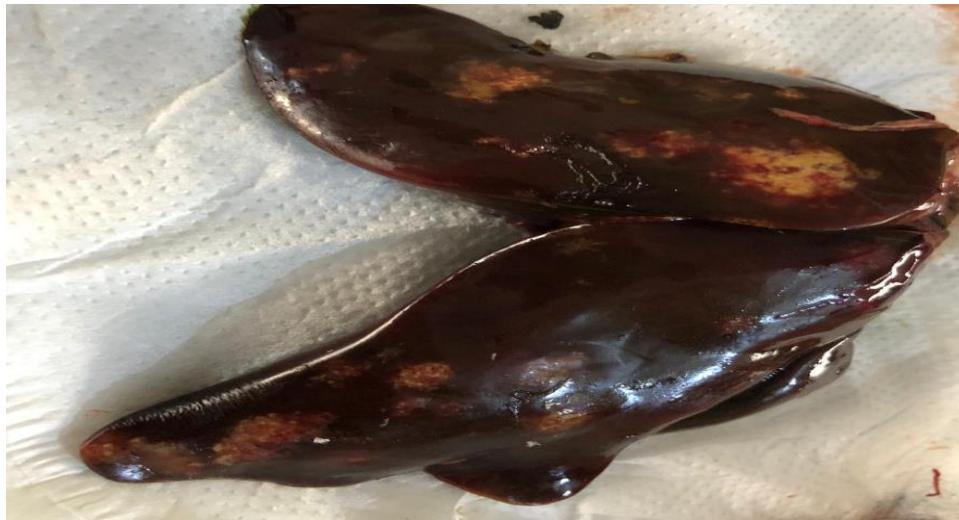


Figure (2): A picture showing the presence of enlargement of the liver and a change in color (dark color) in addition to the necrotic nodes visible on the surface of the liver.

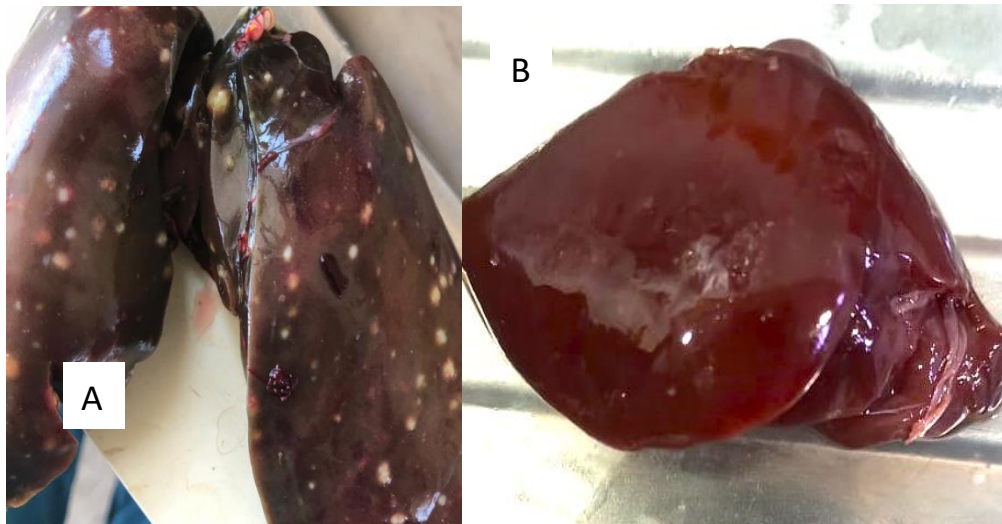


Figure (3): (A) A picture showing the presence of small white nodules (pinpoint size). (B) dark color of the liver with the presence of white nodes of pale color.

3.3 Microscopically

The results of the microscopic examination showed the presence of *H.meleagridis* in (22) liver samples (88%) and (21) cecum samples (84%) as shown in Table (1). This study is the first in Salah al-Din and Kirkuk provinces /Iraq. Microscopic diagnosis of the parasite reveals the presence of the

amoebic form of the parasite in the lesions of liver and cecum, where it appeared in a spherical and oval forms ranging in size between (4-12 μ m) as shown in Figure (4), with the presence of an eccentric nucleus appeared in a dark blue color (basophilic) and sometimes two nuclei showing atypical feature for the parasite (double- eyed)

appearance surrounded by a pale blue to acidic (Eosinophilic) cytoplasm and a wavy plasma membrane with the emergence of pseudopodia in some of them as in Figure (5) and (6).

Table (1): shows the percentage of positive samples of liver & cecum infected with *H. melegridis*.

Total animals samples	positive liver samples		Positive cecum samples	
	Number	%	Number	%
25	22	88	21	84

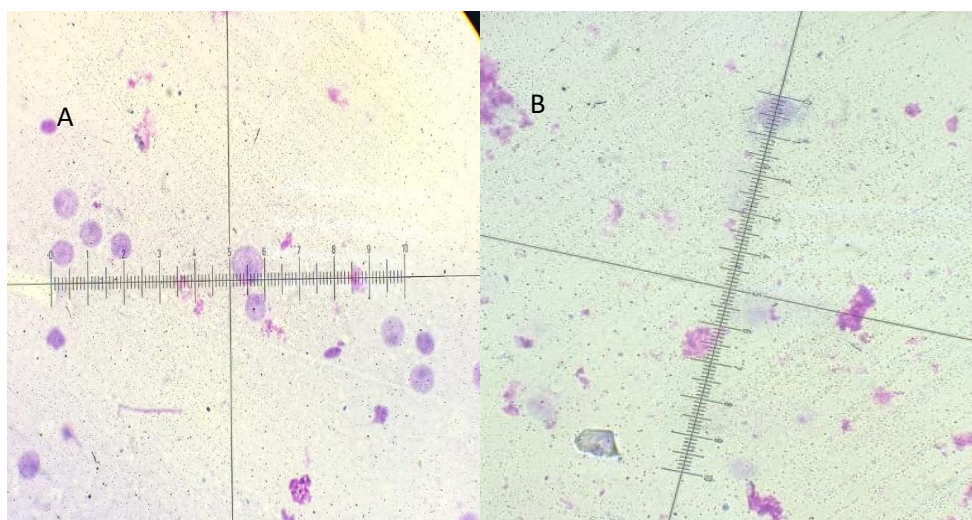


Figure (4):showing the method of measuring the size of the parasite (A) In liver lesions (B) In cecum lesions, stained with giemsa (100 X).

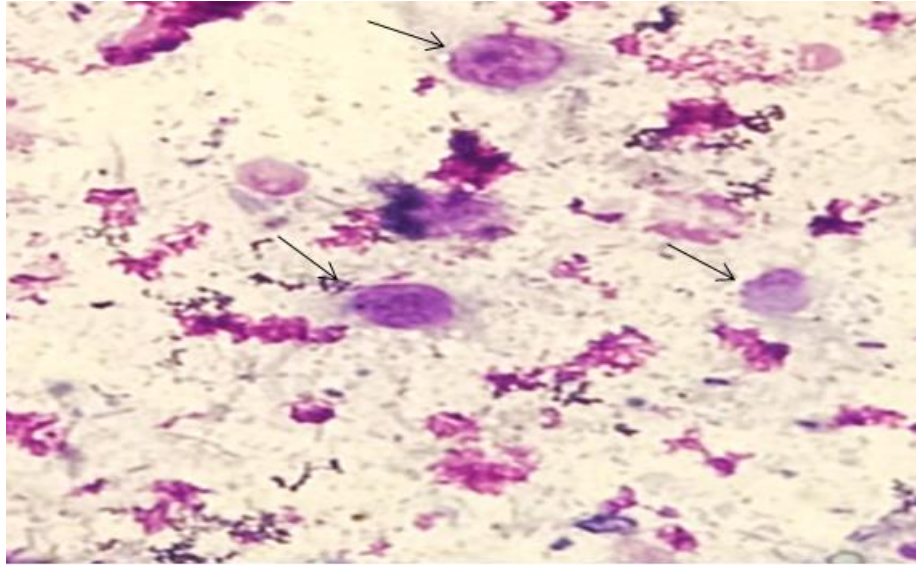


Figure (5): The amoebic shape of the parasite appears in cecum lesions where it appeared in different shapes as an indicator with a black arrow , (100x).

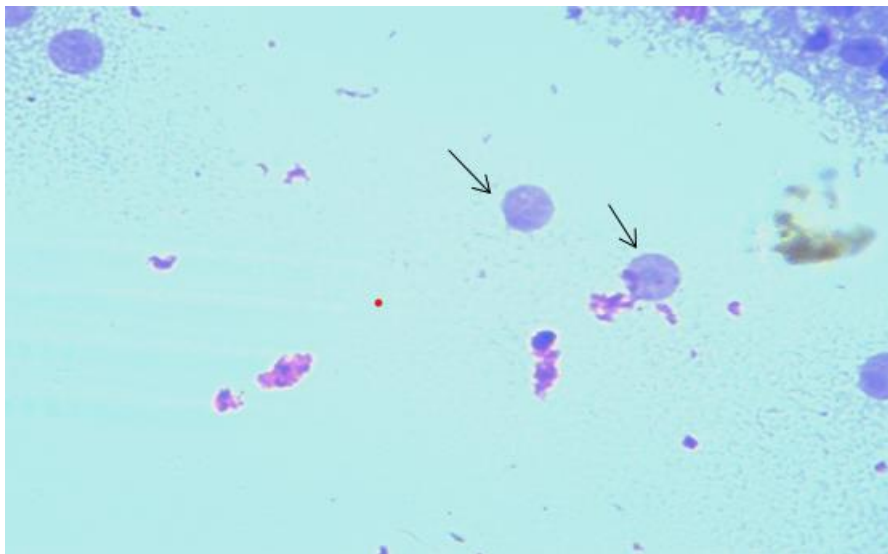


Figure (6): shows the amoebic shape in liver lesions where it appeared in a spherical shape(black arrow) (100x) .

4. Discussions

Histomoniasis, also known as, blackhead disease or enterohepatitis, is caused by the protozoan *Histomonas meleagridis*. The disease mainly affects turkeys and broiler chickens [12]. The disease causes high mortality rates in both Turkeys and chickens; however, turkeys are noted to have a higher mortality rate compared to chickens [13]. Infected Turkeys showing clinical signs such as reduced appetite, sulphur-yellow diarrhoea, dehydration, and emaciation [14].

The current study was aimed to diagnosis of *H. meleagridis* in cecal and liver samples obtained from naturally infected turkeys . Microscopic examination by direct smear method reveals the presence of parasite in (22) liver samples (88%) and (21) cecum samples (84%). The result was agreed with that recorded by [15] in a study conducted in eastern China on 48 liver and cecum chicken samples between 2011 and 2012, where histological diagnosis of liver and cecum samples showed an infectivity rate of 83.33% and 89.58%, respectively. The current study recorded a higher infection rate than what was recorded in previous studies, In Iraq, the percentage was higher than what [16] recorded 0.36% in a clinical

study to diagnose poultry diseases in Nineveh Governorate and higher than what was recorded by [17] 2% in Iraq in a study conducted at Al-Qadisiyah University in Al-Diwaniyah Governorate in the period from September 2014 to the end of June 2015 To isolate and diagnose internal parasites from turkeys . [18] explained that the reason for the high diagnosis of the parasite may be due to the fact that the collection of samples was not random but was collected based on clinical signs that appeared in birds.

Also the results showed the presence of the amoebic form of the parasite in liver and cecum lesions , where it appeared in a spherical and oval shape ranging in size (4-12 μ m) with the presence of an eccentric nucleus that appeared in a dark blue color Basophilic pigment and sometimes two nuclei in a shape similar to the eyes (double eye) and surrounded by a pale blue cytoplasm pigment to acidic Eosinophilic and a wavy plasma membrane with pseudopodia . This agreed with what the Tyzzer stated in 1920 that the tissue or invasive form of the parasite exists in the tissues of the cecum and liver in a amoeboid vegetative form [19]. In Iraq, the results agreed with [8] who diagnosed the parasite with distinctive double-eyed character in swabs taken from liver samples of infected

turkeys in the city of Dohuk in the Kurdistan region of Iraq. The appearance of the parasite with its double-eyed characteristic in the liver is agree with what was reported by [20] in a histological study conducted at Al-Qassim University in the Kingdom of Saudi Arabia on the epidemiology of the parasite in ducks. The results also agreed in terms of shape and are close in size to what was reported by [21], who confirmed in his study that the shape of the parasite in cecal lesions is spherical or almost oval, with a size of (6-10) micrometers.

Grossly, our study revealed the presence of pathognomic lesions for the disease in cecum and liver which agreed with what was mentioned by [22] according to the lesions appeared on the cecum and liver, and also agreed with [14] who noted a liver hypertrophy and lesions that appeared on the cecum. Also agreed with what [3] reported according to the lesions that appeared on the cecum.

The results differed from what was found by [5-14] in terms of the shape and color of the lesions that appeared on the liver, as they stated that the observed lesions on the liver appeared in a form of low circular areas with a green color in the center and raised edges resembling a hole.

The difference in the shape of the lesions from what was recorded in the current study is due to the fact that the diagnosis was in the early stages of the infection, because the lesions at the beginning of the infection appear as small areas of necrosis and inflammation, then they gather as the disease progresses and form larger nodes resembling cancer of the lymph nodes, and in the final stages of the disease and as a result of the acute necrosis of liver cells, this leads to collapse of the visceral tissue of the liver, resulting in the appearance of lesions in the form of circular areas with raised edges [14].

5. Conclusion

The parasite *Histomonas meleagridis* is one of protozoa that infect turkey and chicken . According to the current study , this parasite isolated from turkey for the first time in Salah al-Din and Kirkuk provinces .The infection companied by many signs like depression; yellow diarrhea and a foul smell for some cases, as well as head drooping and irregular feathers and the bluish head was observed only in a few cases at young animals. Macroscopically, the parasite cause a serious tissue changes in liver and cecum

6. References

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تشخيص طفيلي *Histomonas meleagridis* في الديوك الرومية المصابة طبيعياً في مناطق مختلفة من مدينة تكريت وقضاء الحويجة ، العراق

قدامة عبيد¹ ، اميمة محمود²

^{1,2} فرع الاحياء المجهرية، كلية الطب البيطري، جامعة تكريت، العراق

الملخص

تضمنت الدراسة الحالية التشخيص المجهرى لطفيلي *Histomonas meleagridis* بطريقة المسحة المباشرة باستخدام صبغة الكيمزا في عينات الكبد والأعور من 25 عينة من الديوك الرومية المصابة بشكل طبيعي. بينت نتائج الفحص المجهرى وجود الطفيلي في (22) عينة كبد وبنسبة (88%) وفي (21) عينة أعور وبنسبة (84%) حيث ظهر الطفيلي بالشكل الاميبي بشكل كروي او بيضوي وبحجم يتراوح (4_12 مايكرومتر) .

كانت العلامات السريرية للطيور المصابة هي خمول وفقدان الشهية وانخفاض في الوزن مع اسهال اصفر ذو رائحة كريهة لبعض الحالات ،بالإضافة الى تدلي الرأس والريش الغير المنتظم ولم يلاحظ الرأس المزرق إلا في حالات قليلة في الاعمار الصغيرة . اظهر الفحص العياني لعينات الاعور والكبد وجود زيادة في سمك جدران الاعور اضافة الى وجود نزف ومواد متجينة صلبة في تجويف الاعور ، اما في الكبد لوحظ وجود تضخم و بؤر نخريه ذات احجام واشكال مختلفة .

الكلمات المفتاحية: *Histomonas meleagridis*; الديك الرومي ; الكبد ; الاعور