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Trichomonasis detection in pigeons (*Columba livia domestica)* in Diyala province

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Summary

Canker is important disease in Iraq birds and causes a real effects in domestic birds . The grossly lesion in the infected birds were seen white to yellowish mass in different size in the oropharyngeal cavity, as inflammation and ulceration ,the lesion may extended into esophagus ,crop and proventriculus and causes block of the respiratory tract and lead to the death of the birds. Microscopic structures of *T.gallinae* were apparent when smears prepared and stained by Giemsa. the cytoplasm appeared light purple and nucleus with dark purple and clarification of the flagella , nucleus and cytoplasm very well. The results showed a high significant ($P<0.01$) difference between two different ages of infected birds with *T.gallinae* . the adult birds which recorded the highest infection rate 33.33%, while the lower infection rate 20% was among the young birds . females recorded the highest rate 32.30% while the lowest rate 20% was found in the males.The results showed that the higher infection rate with *Trichomonas gallinae* 57.14% was in March among different months of study.

Key words : Trichomonus , pigeons , canker, Diyala.

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Introduction

Avian trichomonaisis is a protozoan disease mainly caused by *Trichomonas gallinae*, which is a mitochondrial anaerobic protozoan, aflagellated parasite belong to the class Zoomastigophorea and order Trichomonadida (1) That infects the upper digestive tract of birds, this parasite infects a widespread of birds, such as pigeons, chickens, turkeys, and other poultry (2,3). Infection by *Trichomonas gallinae* may be without signs or may lead to death, with intermediate symptoms, including: anorexia, vomiting, ruffled feathers ,diarrhea, dysphagia , dyspnea, weight loss, and increased thirst **(**4,5). Several reports indicate that many large events of avian mortality have been associated with *Trichomonaisis* (6,7). The main host of *T. gallinae* is domestic pigeon (*Columba liviadomestica*), and play role in the spread of this disease. Young pigeons are frequently infected by this protozoa and can die from the infection, but adult birds species may act as a carrier without any symptoms (8). Clinical symptoms typically include white to yellowish oral -pharyngeallesions, as inflammation and ulceration progress, the lesions may extend tothe esophagus, crop and proventriculus and this may lead to esophagus obstruction , spreading of the pathogen is possible by penetrating the underlying tissues and inner organs such as the liver (9) **.**

Morphology of *Trichomonas gallinae*

Canker is the common name of the disease in pigeons and doves, also the disease called trichomonaisis (10) . *Trichomonas gallinae* is a single cell flagellated protozoan parasite, infects pigeon in all over world (11). The parasite vary in shape from ovoid to pyriform approximately (6-15) µm long and (4-8)µm wide, its characterized by an undulating membrane that extends two-thirds the length of its body (the undulating appearance is due to the presence of a recurrent flagellum closely attached to the surface) and four free anterior flagella arising from the basal granule and a fifth recurrent one which does not become free at posterior pole (2, 12 , 13).

Prevalence of disease in Iraq

There is a lack of research interests in *Trichomoniasis* present in the pigeon among researchers in Iraq . In a study conducted in Al-Najaf city for isolation of the parasite from the domestic pigeon, and used three methods of isolation, which is the wet swab from the liquid crop of the bird, the swab stained and culture (14). A study conducted in Al- Mosul city to determine the rate of infection and the incidence of *trichomoniasis* in three species of pigeons were included (*Columba oenas*), (*C. livia*), and (*Streptopelia decaocto*), examination of 250, 200 and 40 doves of the three fore-mentioned groups of birds indicated prevalence

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rates of 22%, 17.5% and 10%, for the three species, respectively (**15).** Another study at city of Mosul-Iraq, 100 free living urban pigeons were examined to determine the percentage of infection which was 16% (16). In the city of Al-Diwaniyah a study examined two types of pigeons *Columba livia domestica* and wild pigeon, the results showed that from 130 domestic pigeons 60 birds were positive and 20 positive birds from 65 wild pigeon, the total percent of positive results were 46%, 30.7%, for the tow birds respectively (**17**) . Another study in the same city analyzed the spread of the disease in 100 wild pigeon of *C. livia* and 100 of the *Streptopelia decapcto*, with a total incidence of 29% and 13% respectively (Abd ,2013). Another study collect 128 sample of the easternrock pigeon type *Columba livia gaddi* from different parts of the country to study the effect of infection of *trichomoniasis* on sex, the percentage of infection was 58.5% for males and 71.7% for females (18). A different research was carried out in Babylon province on 138 domestic and wild *Columbide* birds were collected during the period from April to June 2012 ,The result of wet mount technique for *trichomoniasis* was 31.5% (19). another study in Baghdad city on 168 domestic *Columba livia domestica* pigeon, the result showed 58.33 % of the examined pigeons were positive for *Trichomonas gallinae* infection ,the incidence of infection among the examined pigeons were 65.45 % , 62.29 % and 46.15% in adult females, adult male and squabs respectively, seasonally, the highest incidence of infection were 64.1% in spring, 62.4 % in winter, and 61.2 % in summer while the lowest incidence was recorded in autumn 43.2% (20) A study was conducted in Kurdistan region on 249 birds (142 doves and 107 domestic pigeons *Columbids T. gallinae* infection rate was 20.42% in doves and 23.36% in domestic pigeons (21). A total of 609 domestic pigeons were examined in Kirkuk city, Iraq from August 2015 to April 2016, the total infectious rate was 49.26%, distributed to 64.92%, 50.98% and 30.41% in Alzajel, pakistani and zubaeri pigeon breeds respectively, the study compared three different stains to characterize the shape of *T. gallinae* namely Giemsa, Gram and for first time Field's stain , the Giemsa stain was more applicable to stain *T. gallinae* in compare two stains, although Field's stain requires less time for staining compared to giemsa and Gram's stain (22).

Prevalence of the disease in the Arab world:

Several research studies in the regions of the Arabic countries and had examined the incidence of birds with *trichomoniasis*. In Egypt Abd El-Rahman found that infection rate in adult pigeon was 73.5% in females and 69.2% in males while in young pigeon was 72.2% (23). In another study, 288 samples were collected from the pigeons of different ages in Asyut Governorate, when direct examination of the samples was conducted, the total infection rate was 23.2% in the young age group and 57.0% in the adult pigeon (24) In the Saudi Arabia ,the parasite was first time recorded during the examination of 100 pigeons *C. livea domestica* collected in the city of Jeddah ,the total infection rate was 63% , the lesion were seen in the upper gastrointestinal tract, especially in the oral cavity**(** 25) . The

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percentage of infection in the wild pigeons was 68%, hunting bird 35% (26).

Materials and methods

Birds and study area:

A total of 85 pigeons type(*Columba livia domestica*) were brought from the local markets in Diyala province city during the period from the beginning of October 2022 to end of March 2023.The study included sex, months, and the birds were divided into two group the young and adult age group, the sex of birds was determined after doing the anatomy (27).

Laboratory diagnosis:-

Direct smear:-

Oropharyngeal swab samples were taken from the mouths and crop of pigeons randomly by using sterile, pre-moistened, cotton-tipped applicators. It was confirmed by microscopic examination wet mount method (28). *Trichomonas* were identified if motile, flagellated protozoa were observed in the field of microscope (29,30).

Stain Preparation:-

Giemsa stain was prepare as follow:

Giemsa stain solution was prepared by adding 1 part of Giemsa stain solution to 9 part of buffer solution pH7.2 .(31)

Staining parasite:-

Swabs were taken from the oral cavity and crop of suspected pigeons. swab were blended with Phosphate Buffered Saline (PBS) and spread on glass slides ,at that point the slides were fixed with methanol , let it dry atroom temperature then stained with Giemsa stain, stained slides were examined under the oil immersion lens (x100) of the light microscope. Parasites were identified according to (32).

Statistical Analysis

The Statistical Analysis System- SAS program was used to effect of difference factors in study parameters . Chi-square test was used to significant compare between percentage in this study(33)

Results

Gross examination:

The grossly lesion in the infected birds were seen white to yellowish mass in different size in the oropharyngeal cavity, as inflammation and

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ulceration ,the lesion may extended into esophagus ,crop and proventriculus and causes block of the respiratory tract and lead to the death of the birds. Figure(1)

Microscopic examination- Staining examination:

Microscopic structures of *T.gallinae* were apparent when smears prepared and stained by Giemsa. the cytoplasm appeared light purple and nucleus with dark purple and clarification of the flagella , nucleus and cytoplasm very well Figure(2).

Figure(1): Domestic pigeon infected naturally with *Trichomonas gallinae* shows caseous material white to yellowish in color in mouth

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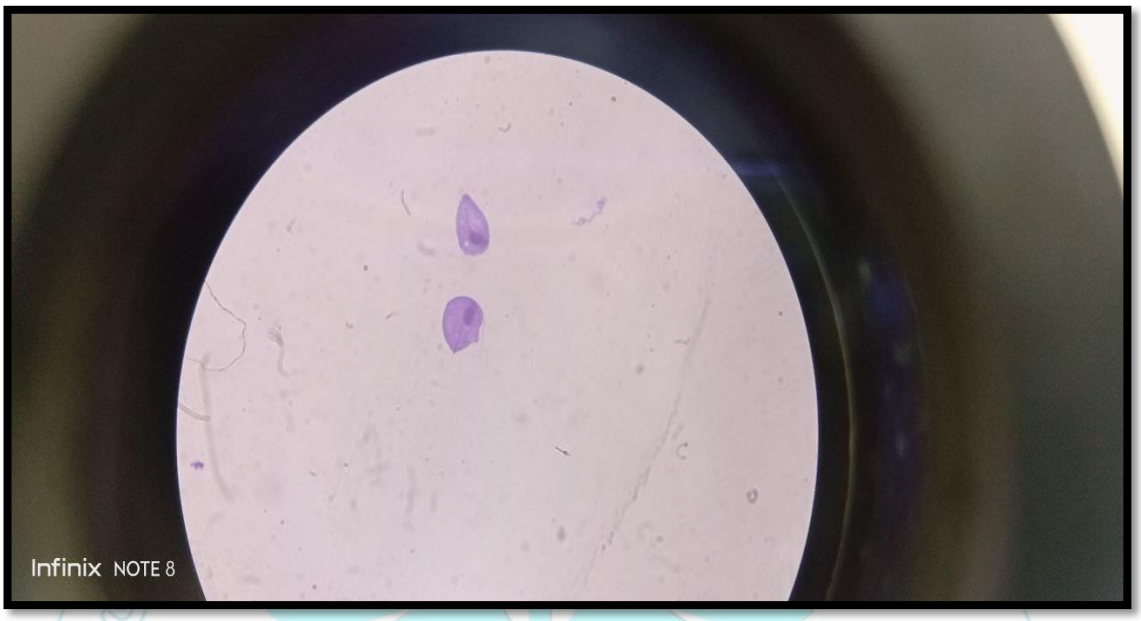

Figure(2):Slide smear of *T.gallinae* .Giemsa stain (x 40)

The total infection rate of *Trichomonas gallinae* was 29.41% (25/85) in Diyala Province during the prevalence study.

1. **The infection rate with *T.gallinae* according to Age.**
   The results showed a high significant (P<0.01) difference between two different ages of infected birds with *T.gallinae* and the adult birds, which recorded the high infection rate 33.33% (20/60) , while the lower infection rate 20% (5/25) was among the young birds(Table1).

Table (1):The infection rate with *T.gallinae* in domestic pigeon based on age

Age	Total number	Positive (%
Young	*25*	*5 (20%)*
Adults	*60*	*20 (33.33%)*
Total	85	*25 (29.41%)*
Chi-Square (χ2)	8.931 **	
**** (P<0.01)**		

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2. The infection rate with *T.gallinae* according to Sex

The results showed no significant difference (P<0.05) between the rate of infection, *T.gallinae* cases were among females, were recorded the highest percentage 32.30% (38/65), while the lowest rate 20% (4/20) was found in the males (Table 2).

Table(2): The infection rate with *T.gallinae* in domestic pigeon based on sex .

Sex	Total number examined	Positive (%)
Male	20	*4 (20 %)*
Female	65	*21(32.30%)*
Total	85	*25 (29.41%)*
Chi-Square (χ2)	8.82	
***** (P<0.01)***		

3. The infection rate with *T.gallinae* according to months:

The results showed that the higher infection rate with *Trichomonas gallinae* 57.14% (8/14) was in Mach (2023) and the lowest 6.66% (1/15) infection rate was in October (2022). with significance difference * (P<0.05) in the infection among different months of study Table (4.3).

Table (3): The infection rate with *T.gallinae* in domestic pigeon based on months**.**

Months	Total number	*Positive (%)*
October (2022)	15	1 (6.66%)
November	17	5 (29.41%)
December	15	4 (26.66%)
January (2023)	14	2 (14.28%)
February	10	5 (50%)
March	14	8 (57.14%)
Total	85	25 (29.41%)
Chi-Square (χ2)	4.209 *	
*** (P<0.05).**		

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Discussion

Grossly examination:

The grossly examination of some domestic pigeons infected with *Trichomonas gallinae* showed lesions in the mouth and esophagus like cheeses material white to yellow in color this results was with agreement to that described by (34,35,36)

Microscopic examination:

The results of using the Giemsa stain in the current study shown good efficiency under microscopic power (x100), Giemsa stain is one of the most suitable dye that used in staining of protozoa and gives good result .The ability of Giemsa stain to show the shape and details of the protozoa has been largely assisted to diagnosis the trichomonaisis, Giemsa stained the nucleus with dark purple and cytoplasm with light purple**.** This agreed with study was done in Kirkuk city which used three different stains to characterize the shape of *T. gallinae* , the giemsa stain was more efficient to stain *T. gallinae* more than other two stains (21) .The disadvantage of thisstain is required aquatic media (BPS) for preparing the dye however, the water media cannot be kept for more than a few hours, so it must be renewed every day (37) .

Study the prevalence of *Trichomonas gallinae* infection in the domestic pigeon (*Columba livia domestica)* recorded in current study 29.41% (25/85) . Which was not in agreement with previous recorded in Iraq by (15) who recorded that the rate of infection was 22% in birds of yamam 17.5% in wild pigeon and 10% in spilopelia senegalensis .In addition (16) recorded 16% in wild pigeons of Mosul. While theprevalence of trichomoniasis was in agreement with (19) also recorded 31.51% in wild pigeons of Babylon**.** Neighboring country showed differ result, in the kingdom of Saudi Arabia, the incidence rate was determined as 50% in domestic pigeon and 68% in wild pigeon (38, 26**).** In Iran recorded 50% in wild pigeons (39). The different in rate of infection recorded by these studies may be due to differ in climatic condition ,geographical region (humidity and temperature) ,season, resistant of the host , change in feeding habit of the birds, age of birds ,number of examined pigeons , size of the sample ,condition of breeding domestic pigeon (40)

The infection rate of *T. gallinae* in the domestic pigeons according to the age in the present study recorded high rate in adult pigeons 33.33% while in young pigeon 20 % which was in agreement with (20) who recorded 63.79% in adult pigeon and 56.92% in young pigeon in Iraq. (41) recorded 50% infection rate in adult columbiformes in Germany .While (37**)** recorded high infection rate in adult pigeons and young aged 72.1% ,64.7% respectively in. The current study results disagree with (42) that recorded 59.50% in adult pigeons and 61.60% in young pigeons in Egypt .Both Al-sadi and Hamodi (16) in Iraq and El-Khatam *et al*

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(35) in Egypt recorded the infection rate in young more than adult .A possible causes of this difference could occur due to the aggregation of adult birds in the feeder and water point as well as stress factor from overcrowding .In addition some type of pigeon have no resistance to the strain of disease, and the way to raise of domestic pigeons in small tower which facilities the transmission of parasite between the birds (43).

There is no significant difference in infection rate of *T.gallinae* infection between female and male 32.30%, 20% respectively. This implies that both sexes share equal chance of getting infected .This result agreement with (37) who recorded an infection rate70.9% in female and 63.8% in male in Bangladesh . Abdel-Rahman *et al*(23) who recorded a higher rate in female 72.36 % and lower rate in male 61.04 % in Egypt .The causes of higher prevalence of *T.gallinae* infection cannot be explained exactly but it is supposed to occur due to female sex hormones that make the individual more susceptible to any infection (44).

The rate of infection with *T. gallinae* in the domestic pigeons according to the months showed that higher infection rate was in March 57.14% and the lower in October 6.66% . This result accordinated with Abdel - Rahman *et al* (23) who recoded highest incidence of trichomonaisis in spring season 87.06 % in Egypt . Jaafar (20) 74% in spring season in Baghdad . These results are nearly closer to that recorded by Eman (42) in Egypt and (45) in Bangladesh . The rate of infection in March is higher because of the natural season of breeding in this month followed by an increase in the number of youngers hatching, which is more sensitive to the disease because of feeding the pigeons milk from mothers contaminated with parasite ,Also climatic factor play a role in spread the disease **(**46).

Conclusion

Canker diseases (*Trichomonas gallinae)* infection is still endemic in Diyala province , the adult pigeons are more susceptible to infection than young.no significant impact of the sex on rate of infection, the season effects appear that high infection rate recoded in spring specially March with significant impact of months in infection rates during the study .

Recommendation

1. Conduct more studies on this type of birds and other species such as Chicken, Turkey ,hunting birds, Pet birds.
2. Use molecular method to know pathogenic and nonpathogenic strain of *T.gallinae*.
3. Try to use some anew medicine in an attempt to treat the parasites and reduce his spread.

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