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Avian Trichomoniasis Prevalence in Domesticated Pigeons in Thi-Qar Province, Iraq

[1]Ahmed Jaafar Mousa, [2]Nothaila Rasheed Hamid

[1,2]Microbiology branch , College of Vet. Medicine, University of Thi-Qar

[1]*Email: Ahmed.vet22@utq.edu.iq*

Abstract

Avian trichomoniasis is a disease that is spread globally in wild and domesticated birds, especially pigeons, caused by the parasite *Trichomonas gallinae* and known as canker. The complications of this illness include lesions in the upper gastrointestinal tract. The present study's goal would be to study the prevalence of *T. gallinae* in domesticated pigeons in Thi-Qar province, Iraq, utilizing the internal transcribed spacer1-5.8s rRNA-internal transcribed spacer2 gene. A total of 405 pigeons including 160 of Alzajil pigeons, 160 of red pigeons and 85 of Arafali pigeons were collected from local markets for the period from September 2022 to May 2023, the infection rates were **38.13, 37.50,** and **42.35** respectively. The study noted a non-significant difference at p. value < 0.05 in distribution of *T. gallinae* according to bird's species. The study found that infection rate is higher in squabs than adults in all pigeons of the three groups. There was no effect of sex on the prevalence rates. As for the months in which the samples were collected the study found there is significant difference at p. value < 0.05 of parasite distribution according to collection months, the highest infection rate was recorded in November 68.89 while the lowest isolated parasite was in April 17.78.

Key words | Thi-Qar province, avian trichomoniasis, pigeons, light microscope**.**

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I. INTRODUCTION

The protozoan *Trichomonas gallinae* causes an important disease in pigeons known as avian trichomoniasis. It is anaerobic, flagellates, and classified in the class Zoomastigophorea and the phylum Trichomonadida [1, 2]. *T. gallinae* primarily affects the digestive and respiratory systems of birds [3]. This disease is usually referred to as "canker" when it affects pigeons [4]. Some strains are virulent, while others are asymptomatic or have mild symptoms. Yellow-green secretions with an unpleasant odor from the mouth, diarrhea, emaciation, severe weight loss, The onset of decaying lesions, the appearance of cheddar-like sores, and oral irritations that prevent swallowing and cause catastrophic respiratory failure are all symptoms of this condition [5]. *T.gallinae* is spread between birds through parents feeding their young and ingesting contaminated food and water. By devouring sick birds, raptors and carnivorous birds are also exposed to it [6, 7]. The parasite can be diagnosed by the following methods: observing the characteristic lesions of the disease; observing the protozoa and its flagella microscopically; culturing the parasite; and using molecular methods [8, 9]. In the subject of molecular epidemiology of microorganisms, polymerase chain reaction and related methods are often recognized as sensitive and reliable methodologies [10]**.** Despite the importance of the parasite, there has been no previous study on it. Therefore, the current study is considered the first in the province of Thi-Qar to deal with the prevalence rates of the parasite and the effect of pigeon type, age, sex, and season on prevalence rates.

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II. MATERIALS AND METHODS

Samples Collection

A total of 405 pigeons including 160 of Alzajil pigeons, 160 of red pigeons and 85 of Arafali pigeons were collected from bird breeders and local markets in Thi-Qar province, Iraq for the period from September 2022 to May 2023.

Microscopic Examination

Using sterile, cotton-tipped applicators that had been previously moistened, samples were randomly obtained from the mouths and pharynx of pigeons (wet mount method). If, under a light microscope, motile and flagellated protozoa were seen, the parasite was recognized [11].

Staining

Randomly selected pigeons had their mouth cavities and pharynx swabbed. The swabs were mixed with 3 ml of phosphate buffered saline, then fixed with methanol, left at room temperature to dry, and then stained with Giemsa [12] which is prepared by adding 1 part of the dye to 9 parts of buffer solution. Under a light microscope equipped with an oil immersion lens (x100), the slides were inspected. According to [13], the parasite was detected.

DNA Extraction

Using the g SYNCTM DNA Extraction Kit from Gneaid UK, in accordance with the manufacturer's protocol, genomic DNA was extracted from each isolate of *Trichomonas gallinae*. Until PCR analysis, samples were kept frozen at a temperature of -20 ˚C.

Real Time PCR

Table 1 lists the ITS1–5.8s rRNA–ITS2 gene primer sequences. Sample DNA (3 µL), forward and reverse primers (1 µL each), syber green master mix (12.5 µL), and sterile deionized water make up the 20 µL RT-PCR reaction mixture. The qPCR procedure was performed in triplicate. The qPCR program began with initial denaturation for 3 minutes at 94 ºC, followed by 40 cycles, including 30 seconds at 94ºC, 1 minute at 60 ºC, and an extension of 30 seconds at 72 ºC.

Table 1: Primer sequences used for real time PCR

Gene	Primer Sequences (5´- 3´)	The Size of the item	source
ITS1-5.8s rRNA-ITS2	F:CCTGCCGTT GGATCAGTTCT R:AGGAGCCAA GACATCCGTTG	372bp	[14]

Statistical Analysis

The current data were statistically analysis by using SPSS statistical software version 26, based in using Chi-Square and Independent sample t test at p. value < 0.05.

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III. RESULTS AND DISCUSSION

Occurrence of *T. gallinae* in Pigeons

According to this investigation's findings, the protozoan infection rates were **38.13, 42.35, 37.50** in Alzajil, Arafali and red pigeons, respectively. The findings of the present study were close to those recorded in Babylon province of Iraq (38.88%) by [15] in rock pigeons, in United states (36%) in a declining band-tailed pigeon by [16], but higher than that recorded by some researchers such as [17], which recorded 16% infection rate in wild pigeons of Mosul of Iraq, [18] which recorded 26.6% in domestic pigeons of southern China, [19] which recorded 26.85% in Columba livia domestica in Assam, India, [20] which recorded 27.5% in domesticated pigeons in southern Ukraine, and lower than the that recorded by some researchers ,such as [21] which recorded 58% infection rate in columba livia domestica in Baghdad province. In neighboring countries, it was found that the infection rate was 50% in the domesticated pigeons in Saudi Arabia [22], and 68% in wild pigeon [23], in Iran recorded 50% in wild pigeons [24]. The climatic conditions, geographical region (humidity and temperature), season, host resistance, change in feeding behavior of the birds, age of the birds, number of examined pigeons, size of the sample, and breeding domestic pigeon conditions, examination methods, could all be factors in the different infection rates observed in these studies [25, 26].

Gross examination

The oropharyngeal cavity of infected birds showed a gross lesion that ranged in color from white to yellowish and was of varying size. As a result of inflammation and ulceration, the lesion may have spread to the esophagus, crop and proventriculus, larynx, blocking the respiratory tract and ultimately killing the birds (Figure1). This is consistent with some local studies, such as: the study conducted by [27] in AL-Mosul, another study conducted in the city of Mosul by [17], [28] in Baghdad , also agree with [5] in Egypt, [29] in Bangladesh, [30] in Pakistan, [31] in Austria, [32] in Germany, [33] in Mauritius. The lesion which showed in current study represent the host's reaction to the presence of parasite which including made up of blood cells (mainly leucocytes), tissue remnants, parasitic degenerates, and other substances [34].

Microscopic examination- Staining examination:

The jerky movement of the parasite was seen in the motile creature when it was viewed under a microscope at x40 or higher. The parasite was recognized based on its distinctive characteristics. It has an oval or pear-shaped form, four anterior flagella, one undulating membrane, and a conspicuous axostyle at the posterior end of the parasite (Figure 2).Then Giemsa stained the nucleus with dark purple and cytoplasm with light purple. This is consistent with many studies such as: The 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 [35], also agreed with [36] in Diyala Province, [37] in Al-Qadisiyah Province, [5] in Egypt, [38] in Iran , [39] in United States.

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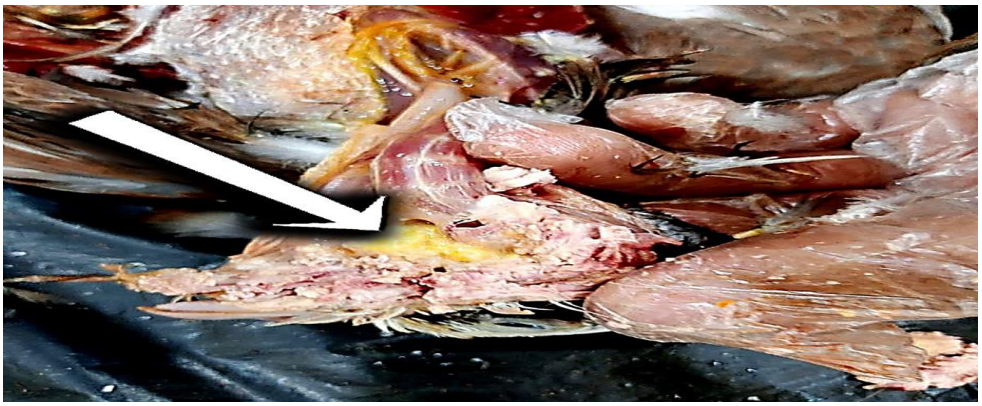

Figure (1): domestic pigeon infected with *T.gallinae* shows caseous material white to yellowish in color in the oropharyngeal cavity (white arrow).

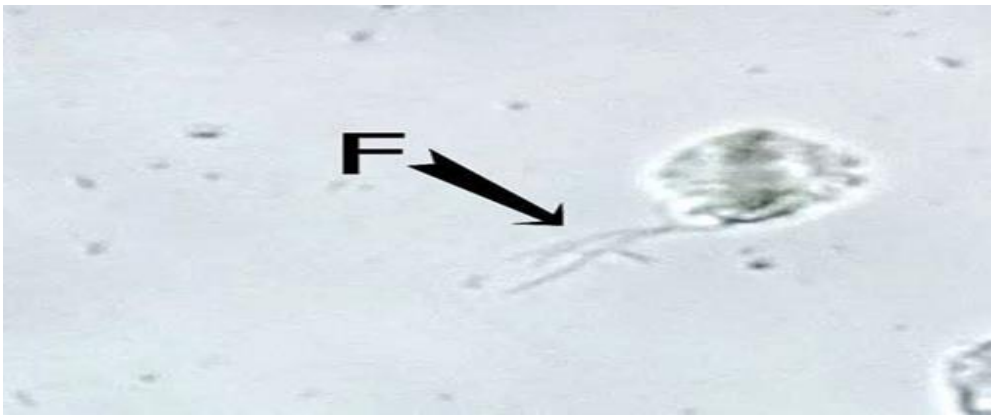

Figure (2): *T. gallinae* in direct wet smear from domestic pigeon (x40).

Infection Rate of *T. gallinae* parasite according to Species of Birds

The present study recorded the rate of parasite infection was non-significantly increased in Arafali Pigeons 42.35%, while decrease non-significantly in Red pigeons 37.5%, the results also noted a non-significant difference at p. value < 0.05 in distribution of *T. gallinae* according bird's species as in table (2). This results agreed with the findings of [40], where the researcher studied the parasite in two types of birds that included domesticated pigeons and Doves in some villages in the Kurdistan Region of Iraq, the researcher did not notice a significant difference between the affected species. The current study does not agree with the study conducted by [27] in the city of Mosul, [35] in Kirkuk province. The absence of a significant difference between the species studied in the current study may be due to the fact that all of these species are domesticated birds that share the same food nature, receive the same care and breeding conditions, and may share the same cages, so the rates of parasite infection were close, although Alzajil pigeons are raised for the purpose of racing while the main purpose of Breeding, while Arafali and Red pigeons for entertainment and decoration.

Table (2): Infection rate of T.gallinae according to bird species.

Birds	Infected		Non-infected		Total	
	No.	%	No.	%	No.	%

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Alzajil pigeons	61	38.13	99	61.88	160	39.51
Arafali Pigeons	36	42.35	49	57.65	85	20.98
Red pigeons	60	37.50	100	62.50	160	39.51
Total	157	38.77	248	61.23	405	100
$CalX^2$= 0.495	$TabX^2$ = 5.99	DF=2	p. value 0.781			

Infection Rate of *T. gallinae* parasite according to Age of Birds

The current study showed a non-significant difference at p. value < 0.05 of parasite distribution in Alzajil pigeons and Red pigeons according to age groups, also within same group, noted the infection rate increased non-significantly in Squabs 50.0% and 42.86% respectively. In contrast Arafali Pigeons scored a significant difference at p. value < 0.05 was noted significantly in Squabs 57.14%. The current study also noted a non-significant difference between species of bird according to age categories as in table (3). This results agree with [27] in the city of Mosul , [26] in Egypt, also with [7] in Bangladesh, [41] in China, , while disagree with [21] in Baghdad city. It is noted by extrapolating the results that (squabs) are more infected than the adult birds of the three types of birds in this study, and the reason for this may be attributed to the direct transmission of infection from mothers to squabs during feeding (crop milk).

Table (2): Infection rate of *T.gallinae* parasite according to age groups.

Birds	Age	Infected		Non-infected		Total	
		No.	%	No.	No.	%	No.
Alzajil pigeon	Squabs	3	50.0	3	50.0	6	3.75
	Young	18	35.29	33	64.71	51	31.88
	Adults	40	38.83	63	61.17	103	64.37
Total		61	38.13	99	61.87	160	100
$CalX^2$= 4.976		$TabX^2$ = 5.99		DF=2		p. value 0.083	
Arafali Pigeon	Squabs	4	57.14	3	42.86	7	8.24
	Young	6	42.86	8	57.14	14	16.47
	Adults	26	40.63	38	59.38	64	75.29
Total		36	42.35	49	57.65	85	100
$CalX^2$= 6.102		$TabX^2$ = 5.99		DF=2		p. value 0.047	
Red pigeon	Squabs	6	42.86	8	57.14	14	8.75
	Young	6	31.58	13	68.42	19	11.87
	Adults	48	37.80	79	62.20	127	79.38
Total		60	37.50	100	62.50	160	100
$CalX^2$= 2.584		$TabX^2$ = 5.99		DF=2		p. value 0.275	
Between Squabs	DF=2 $TabX^2$ = 5.99					p. value 0.141	

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CalX^2= 3.920		
Between Young CalX^2= 2.785		**p. value 0.248**
Between Adult CalX^2= 0.196		**p. value 0.907**

Infection Rate of *T. gallinae* parasite according to Sex of Birds

The current study showed a non-significant difference at p. value < 0.05 of parasite distribution in Alzajil pigeons and Red pigeons according to sex, also within same group, noted the infection rate increased non-significantly in male than female 41.51% and 39.74% respectively. In contrast Arafali Pigeons scored a significant difference at p. value < 0.05 was noted significantly increase in male 50%. The current study also noted a non-significant difference between species of bird according to sex categories as in table (4). In general, in view of the above, it can be considered that there is no effect of sex with regard to infection with T.gallinae. This agree with [42] in Baghdad , [40] in Kurdistan , [21] in Baghdad. This result disagree with [27] in the city of Mosul. According to the current study, the percentage of parasite infection was higher in all studied species, as the difference was non-significant between the sexes in both Alzajil and Red pigeons, while significant in Arafali Pigeons, this may be due to the small number of samples collected from Arafali compared to other species, given that there are fewer breeding projects in the province.

Infection Rate of *T.gallinae* according to Collection Months

The current study showed a significant difference at p. value < 0.05 of parasite distribution according to collection months, the high infection rate was recorded in November 68.89, followed in October 57.78%, followed in May 51.11%. In contrast the lowest isolated parasite was in April 17.78 as in table (4). This results closer to [27] in Al-Mosul city, Which recorded the presence of a seasonal effect of the disease, as the rate of infection in all types of birds increases in the winter and spring seasons more than the rest of the seasons of the year, [42] in Baghdad city which recorded highest prevalence during spring and winter and lowest prevalence during Autumn, [19] in India which found highest prevalence during winter, [29] in Bangladesh which recorded that T. gallinae infection was significantly lower in summer than rainy and winter seasons. This study disagree with [43] in China which showed that high prevalence in the months of July and August, [44] in Mauritius which noted that *T. gallinae* infection prevalence is higher at sites and times of warmer temperatures and lower rainfall. It is noted by extrapolating the results that the highest infection rate was in November followed in October followed in May. This may be attributed to the low temperatures and the increase in relative humidity, as the parasite cannot live for a long time outside the body and at high temperatures, even after the death of the bird [45].

Table (4): Infection rate of *T.gallinae* according to sex.

Birds	Sex	Infected		Non-infected		Total	
		No.	%	No.	%	No.	%
Alzajil pigeon	Male	22	41.51	31	58.49	53	51.46
	Female	18	36.0	32	64.0	50	48.54
Total		40	38.83	63	61.17	103	100

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CalX²= 0.757		**TabX² = 3.84**		**DF=1**	**p. value 0.384**		
Arafali Pigeon	Male	19	50.0	19	50.0	38	60.32
	Female	7	28.0	18	72.0	25	39.68
Total		26	41.27	37	58.73	63	100
CalX²= 10.172		**TabX² = 3.84**		**DF=1**	**p. value 0.001**		
Red pigeons	Male	31	39.74	47	60.26	78	59.09
	Female	20	37.04	34	62.96	54	40.91
Total		51	38.64	81	61.36	132	100
CalX²= 0.190		**TabX² = 3.84**		**DF=1**	**p. value 0.663**		
Between Male CalX²= 2.237	**DF=2 TabX² = 5.99**		**p. value 0.321**				
Between Female CalX²= 2.179			**p. value 0.336**				

Extraction of genomic DNA

T. gallinae DNA was extracted from 100 swabs of the mouth, pharynx by the previously mentioned method (Figure 3).

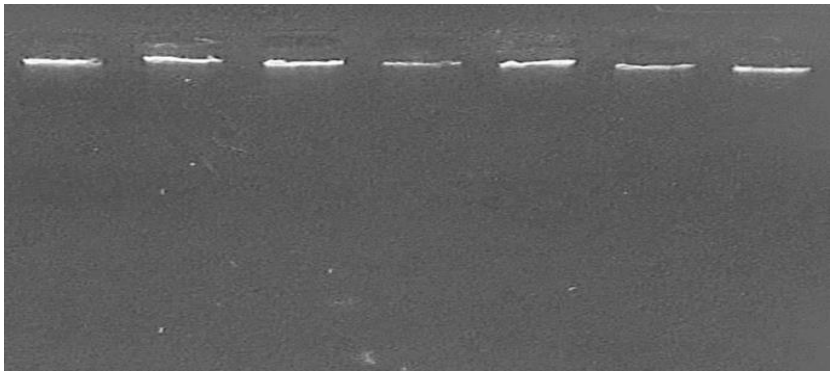

Figure (3): Agarose gel electrophoresis of genomic DNA in T. gallinae before PCR.

Table (4): Infection rate of *T.gallinae* according to collection months.

Birds	Infected		Non-infected		Total	
	No.	%	No.	%	No.	%
September	9	20.00	36	80.00	45	11.11
October	26	57.78	19	42.22	45	11.11

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November	**31**	**68.89**	**14**	**31.11**	**45**	**11.11**
December	**12**	**26.67**	**33**	**73.33**	**45**	**11.11**
January	**12**	**26.67**	**33**	**73.33**	**45**	**11.11**
February	**9**	**20.00**	**36**	**80.00**	**45**	**11.11**
March	**22**	**48.89**	**23**	**51.11**	**45**	**11.11**
April	**8**	**17.78**	**37**	**82.22**	**45**	**11.11**
May	**23**	**51.11**	**22**	**48.89**	**45**	**11.11**
Total	**152**	**37.53**	**253**	**62.47**	**405**	**100**
CalX^2= 56.575 **TabX^2 = 15.51** **DF=8** **p. value < 0.001**						

Real-time PCR

Total samples 100 were tested by using RT-PCR ITS1-5.8s rRNA-ITS gene the results showed 95% were positive. This consistent with the high percentages recorded by many local, regional and international studies, such as [21] which recorded (85%) in Baghdad city, [37] which recorded (100%) in Al-Qadisiyah Province, also consistent with [46] which recorded (100%) in KSA, [47] which reported (100%) in Britain. By PCR we were able to differentiate Trichomonas spp. which are difficult to differentiate by microscopy. Polymerase chain reaction (PCR) has been used to diagnose Trichomonas disease in pigeons, as well as to differentiate Trichomonas spp.

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