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Prevalence and Pathology of *Trichomonas gallinae* in Domestic Pigeon (*Columba livia domestica*) of Assam, India

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ABSTRACT

Background: *Trichomonas gallinae* is a flagellated protozoa, infects the upper digestive system of pigeon and causing a condition called canker, characterized by development of granulomatous lesions in the buccal cavity, crop, proventriculus, gizzard and liver of affected birds causing mortality upto 90% in squabs. Systemic research on prevalence and pathology of *T. gallinae* in pigeons are scant in India. The present work was carried out to document the prevalence and pathology of *T. gallinae* in pigeons of Assam.

Methods: During the one- year study period, throat swab samples were collected from households, market places and temple premises in 4 districts of Assam. A total of 324 birds were examined by wet mount and Giemsa staining methods for detection of *T. gallinae*. Naturally died carcasses were studied for gross and histopathological alteration in different organs.

Result and Conclusion: In the present study prevalence of *T. gallinae* was found 26.85% and squabs were highly susceptible with 56.25 percent. Sex wise, comparatively higher prevalence was observed in females (33.54%) than in the males (20.48%). Seasonality study revealed highest prevalence during winter. Gross pathological alteration characterized by yellowish white caseo-necrotic haemorrhagic lesion in the upper digestive tract beginning with the oral cavity and lesions also found in liver and lungs. Histopathological alteration also recorded in different organs. In the liver sinusoidal congestion and kupffer cell hyperplasia were observed. Infiltration of polymorphonuclear cells in the thickened inter alveolar space of lungs also recorded. Further study is required to identify the prevalent strains of *T. gallinae* on the basis of pathogenicity in this geographic region and immunology of *T. gallinae* in pigeons and other hosts remain to be explored.

Key words: Pathology, Pigeon, Prevalence, *Trichomonas gallinae*.

INTRODUCTION

Pigeon rearing is very popular in rural and urban areas from Assam and other states of Northeast India. Raising of local native pigeon with minimum investment is practised within the home yards by all sections of society for production of pigeon squabs for meat which is highly nutritious, delicious and also restorative for the convalescents. Besides family nutrition among the non vegetarian societies of the states Kumar *et al.* (2007), people release young pigeons in pair in the temples and other religious places during prayer for fulfillment of their wishes Roy *et al*. (2011). Thus pigeon squabs are highly demanding and provide supplementary income to the households.

Pigeons are affected with several health problems which cause retardation in growth, lower egg production and at times death especially in squabs Dranzoa *et al*. (1999). Among parasitic causes *T. gallinae* a flagellated protozoa infects the upper digestive system of pigeon and cause canker, a condition characterized by development of granulomatous lesions in the buccal cavity, crop, proventriculus, gizzard and liver of affected birds. The parasites get transmitted to the nestlings through feeding of pigeon milk by the infected parents (Soulsby 2012). Morbidity and mortality in pigeons varies with the different strains of the parasites. A highly virulent strain can cause 50% mortality in adults while it may reach up to 90% in the squabs (McDougald, 1992). Literature on prevalence and pathology of spontaneous cases of *T. gallinae* in pigeons are scant in India and no systematic research work has been done so far. In view of high demand for pigeon meat and commercial prospect of pigeon farming in the region, the present work was carried out to document the prevalence and pathology of *T. gallinae* in pigeons of Assam.

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MATERIALS AND METHODS

Study area

The study was carried out for one year (March 2015 to February 2016) by collecting throat swabs of pigeons from various households, market places in 4 districts of Assam, namely Kamrup Rural, Kamrup Metro, Lakhimpur and

Dhemaji and temple premises of Kamakhya and Navagraha temples situated at Guwahati, Assam.

Sample collection

A total of 324 pigeons were included for the study. The oral cavity/oropharynx of each bird was grossly examined for the presence of white, caseous lesions or stomatitis as an indicator for trichomoniosis and the observation was recorded. The pigeons were categorized age wise into squab (< 30 days), young (30-90 days) and adult (> 90 days) and sex wise (male and female).One year study period was divided into four seasons *viz.* Pre-monsoon (March, April, May), Monsoon (June, July, August, September), Post-monsoon (October, November) and Winter (December, January, February).

Examination procedure

Fresh swabs from throat/oropharyngeal region/crop were collected aseptically in normal saline solution (NSS) from each bird with or without showing lesions. Entire wall of the crop was contacted in a swirling motion with the sterile swab and the fluid soaked cotton swabs were examined under light microscope (X400) by preparation of wet mount and Giemsa stained smear (X400, X1000) for the presence of pear shaped trophozoites of *T. gallinae*.

Pathological study

A total of 55 carcasses of domestic pigeons that died naturally during the present study formed the samples for post mortem examination. Each carcass was examined thoroughly for presence of gross lesions and any alteration observed due to *T. gallinae* infection was duly recorded.

Histopathological study

For histopathological examination, representative tissue samples showing typical lesion of *T. gallinae* infection were collected from different organs *viz*. trachea, oesophagus, crop, gizzard, lungs, liver and heart. The samples were preserved in 10% formalin solution. Representative pieces of tissues were cut into 2-3 mm thickness and kept for further fixation. Tissues were washed in tap water overnight, dehydrated in ascending grades of alcohol and cleared in xylene and then embedded in paraffin. The paraffin embedded tissues were cut into sections of 4-5 micron (µ) in thickness and stained with routine Haematoxylin and Eosin method following standard procedure of Luna (1968).

Analysis of data

Chi-square test was used for statistical analysis of the prevalence data by using SAS v.20 software.

RESULTS AND DISCUSSION

In the present study, microscopic examination of 324 throat swab samples showed detection of *T. gallinae* (Fig 1) in 87 cases with a prevalence of 26.85%. Similar findings were shown by workers as Bahrami *et al.* (2012) from Iran (26.8%), Tasca and Carli (1999) from Brazil (26.5%), Al- Sadi and Hamodi (2011) from Iraq (16%) and Qiu *et al.* (2012) from China (33.9%). The reason for such moderate infection might be due to transmission of the parasite by adults while feeding their young or in feeder and water as adult birds remain infected for a year or more acting as a constant source of infection for their young ones. However, higher infection rates (43-75%) have been reported by many workers: Saleem

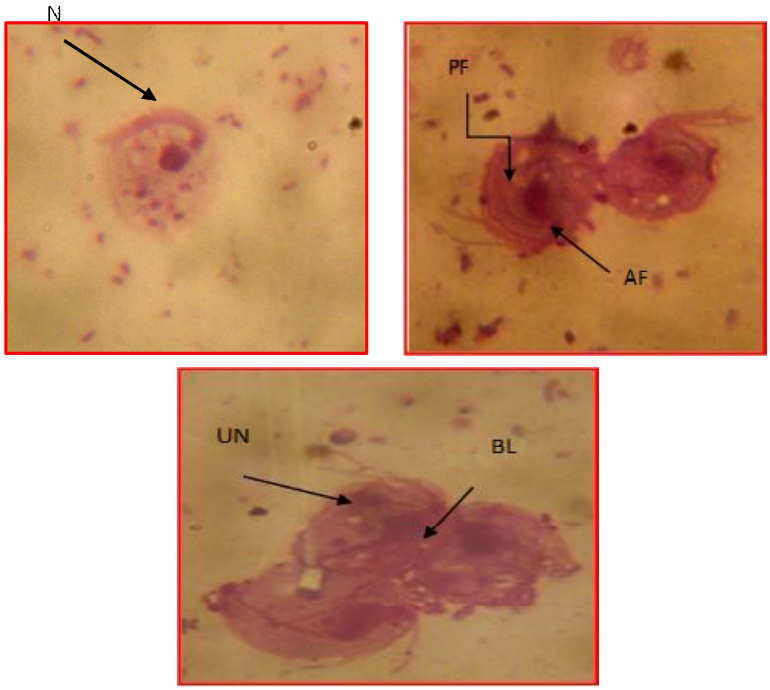


Fig 1: Trophozoites of *Trichomonas gallinae* in throat swab smear (X1000); N-nucleus, AF-anterior flagella, PF-posterior flagella, BL-blepharoplast, UN-undulating membrane.

et al. (2008) from Lahore, Pakistan; McKeon *et al.* (1997) from Australia; Begum *et al*. (2008) from Bangladesh. The differences in the rate of prevalence might be due to influencing factors of disease occurrence such as climatic conditions, geographical difference, seasonal variation, resistance of the host, different feeding habits, difference in husbandry practices as mentioned by Saleem *et al*., (2008) and Hafidth *et al*., (2011).

Examination of Giemsa stained swab smears showed single celled, oval to pyriform flagellated protozoan approximately 6.3-15.5 µm long and 4.0-8.3 µm wide with four anterior flagella (7-13 µm in length) arising from the basal granule lying at the anterior end of the body. The parasite showed an undulating membrane formed by a flagellum closely attached to the body surface and extended to two-thirds the length of its body. The ovoid nucleus (2.5-3 µm diameter) was found close to the base of the anterior flagella. The axostyle originating at the pole opposite the nucleus was seen running from the base of the anterior flagella to the posterior end of the cell and protruding from it. Trophozoites of *T. gallinae* were found to be morphologically identical to those reported by Melhorn *et al.* (2009) and Amin *et al.* (2010).

Prevalence of *Trichomonas gallinae* infection according to age group

Prevalence was found highest (56.25%) in squab followed by 22.38% in young and least in adult (10.90%) (Table1). Statistically, the percentage prevalence of *T. gallinae* in different age group was significant (P<0.05) by Chi square analysis.

The prevalence of trichomoniosis was more in squabs as compared to the adult pigeons which is in accordance with the report of other workers (McDougald, 2003; Al-Sadi and Hamodi, 2011). The main route of transmission of the organism to the squabs is through feeding of “crop milk” which is a mixture of the secretion from the crop glands and regurgitated food containing this pathogen fed by the carrier adult parent pigeons. The present findings are in agreement with that of Hafidth *et al*. (2011), Begum *et al.* (2008), however reported non significantly highest prevalence of *T. gallinae* in adult followed by squabs and young birds. The highest prevalence in squabs followed by young and least in adults as observed in the present study might be related to the resistance status that reflects the age of the host to have an effect on the prevalence of *T. gallinae* in pigeons.

Prevalence of *Trichomonas gallinae* infection according to sex of pigeon

Sex wise, 53 out of 158 samples examined from female birds were positive (33.54%) while in males, the corresponding value was 20.48% (Table 2). Comparatively higher prevalence was observed in female than in male birds and this variation was also found statistically significant (P<0.05). Similar finding was also shown by Begum *et al.* (2008). However, Al- Sadi and Hamodi (2011) and Hafidth *et al.* (2011) recorded higher infection in male than in female pigeons. Villanua *et al.* (2006) on the other hand reported

Table 1: Age wise prevalence of *Trichomonas gallinae* infection in pigeon.

Age group	No. of pigeon examined	No. of pigeon positive	% Prevalence	Chi square value	P value
Squab (<30 days)	80	45	56.25	8.66	0.0131*
Young (30-90 days)	134	30	22.38		
Adult (< 90 days)	110	12	10.90		
Total	324	87	26.85		

*Significant (P<0.05).

Table 2: Sex wise prevalence of *Trichomonas gallinae* in pigeon.

Sex	No. examined	No. positive	% Positive	Chi-square value	P value
Female	158	53	33.54	5.8652	0.0154*
Male	166	34	20.48		
Total	324	87	26.85		

*Significant (P<0.05).

Table 3: Seasonal prevalence of *Trichomonas gallinae* in pigeon.

Month/season	Samples screened for *T. gallinae*	Samples positive for *T. gallinae*	Chi-square value
Premonsoon (March, April, May)	90	23(25.55)	6.6119^{NS}
Monsoon (June, July, August, September)	78	18(23.07)	
Post monsoon (October, November)	56	12(21.44)	
Winter (December, January, February)	100	34(34.0)	
Total	324	87(26.85)	

^{NS}(Non-significant) (P>0.05).

non significant difference in prevalence of *T. gallinae* according to sex. The cause of higher prevalence in females could not be justified although Lloyd (1983) assumed that female sex hormones might play a role making the individual more susceptible to any infection.

Seasonal prevalence of *Trichomonas gallinae* in pigeon

Clinical and subclinical illness in pigeons were recorded throughout the year. Season wise, prevalence was found highest in winter (34.0%) followed by Pre-monsoon (25.55%), Monsoon (23.07%) and Post-monsoon (21.44%) showing no significant difference (Table 3). However, Begum *et al*. (2008) recorded higher prevalence in rainy and winter seasons than in summer. Thus, wide variation could be noticed in different studies on the prevalence of *T. gallinae* depending on season, host factor and other epidemiological factors such as climate, geographical region, host resistance and living status of birds as opined by other workers (Saleem *et al.* 2008; Hafidth *et al*. 2011; Amin *et al.* 2014).

Gross pathological findings

In the present study trichomoniosis was diagnosed in 14 out of 55 carcasses at post mortem examination. External findings included emaciated body with ruffled feather, swollen beaks, puffed up appearance of mouth, exudation of foul smelling fluid through mouth with the beaks wide apart. In some cases lesions around the eyes were seen with appearance of a whitish membrane partially covering the eyes (Fig 2a). Further examination of carcasses revealed presence of yellowish white caseated masses with lesions in the oral cavity (Fig 2b-c), oesophagus, crop (Fig 2e) and proventriculus while the gizzard had yellowish to greenish areas of necrosis. In the liver circumscribed areas of necrosis were seen (Fig 2d). Our findings confirmed the observations made by several workers (Begum *et al*. 2008; Sansano- Maestre *et al*. 2009; Borji *et al*. 2011; Al-Sadi and Hamdi 2011). According to Neimanis *et al*. (2010) presence of gross lesions is a primary diagnostic feature of this disease. The lesions in different organs were basically inflammatory, ulcerative and necrotic in nature as similar to those observed in experimentally induced trichomoniosis (McDougold 2003 and Hafidth *et al*. 2011) reported absence of a secondary disease as a pre-requisite for the occurrence of *Trichomonas* infection. Isolation of facultative bacterial pathogens from natural cases of trichomoniosis with caseated lesions rather revealed association of secondary infection (Mohamed *et al*. 2009) that produced caseative necrosis with drooling of offensive odour fluid from mouth under the stress of trichomoniosis. Although no attempt was made to isolate bacterial pathogens in the present study, similar observation on the presence of caseated mass inside oral cavity and drooling of offensive exudate through mouth might suggest secondary bacterial infection as was reported in earlier studies (Mohamed *et al*. 2009). Observation of grossly visible lesions in the eyes of affected pigeons agreed with Bondurant and Honingberg (1994) who reported that the parasite could invade the brain and the eye region with release of organism in tears.

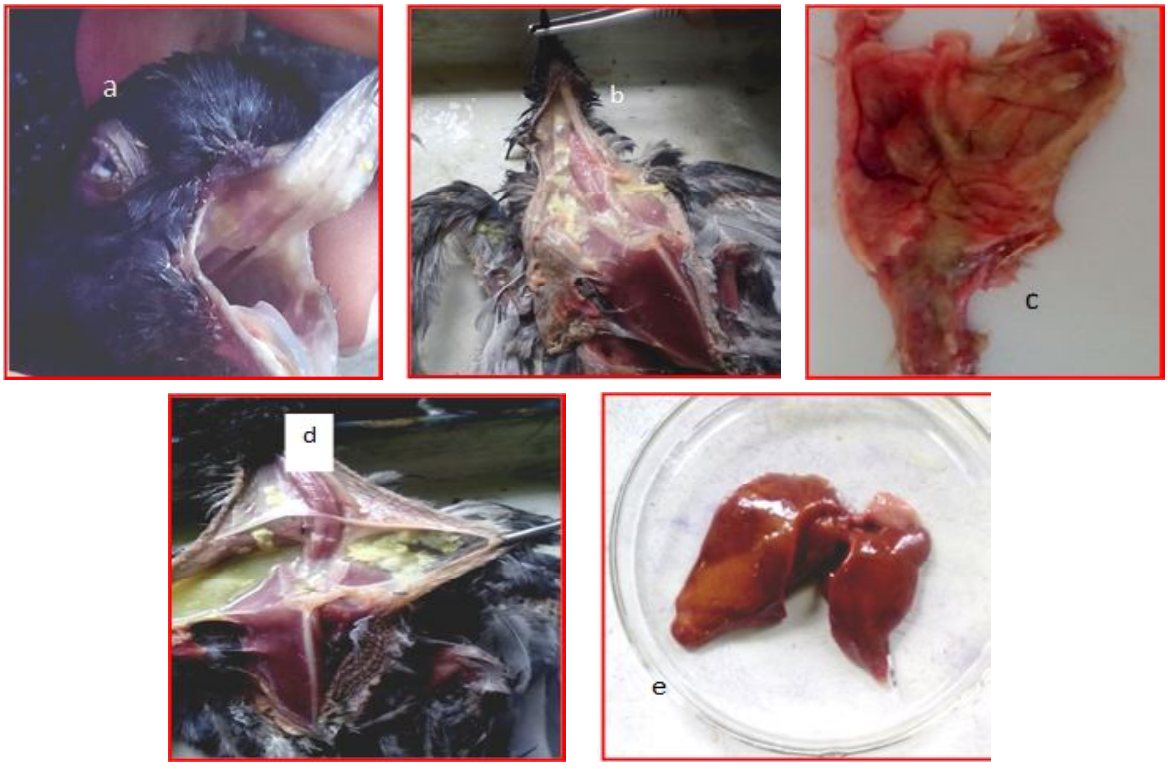


Fig 2: Gross pathological lesions seen in pigeons due to *T. gallinae* infection, (a) Whitish membrane partially covering the eyes, (b-c) Yellowish cheesy exudates in the crop(d) Pale necrotic areas in the liver, (e) Haemorrhagic lesion in the crop.

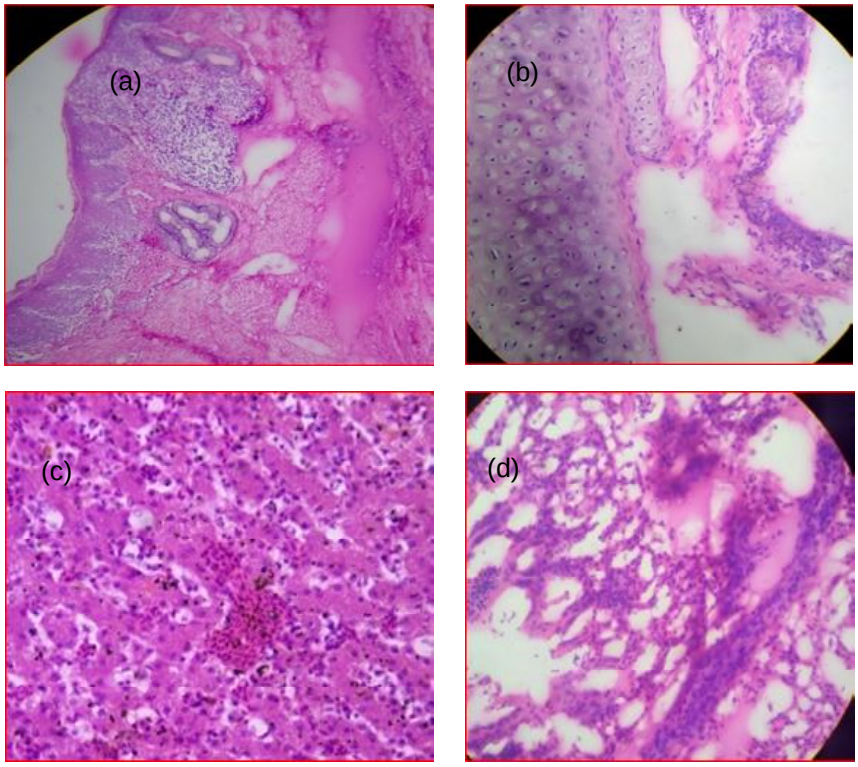


Fig 3(a): Oesophagus showing swollen submucosal glands H&E X100, **(b)** Trachea showing sloughing of mucosal layer H&E X100 **(c)** Liver showing sinusoidal congestion and kupffer cell hyperplasia H&E X 400 **(d)** Lung showing infiltration of polymorphonuclear cells in the thickened inter alveolar space H&E X400.

Histopathological findings

Microscopically, histopathological lesions were observed in the crop, oesophagus, gizzard, liver, heart, trachea and lungs (Fig 3 a, b, c, d). Crop showed moderate haemorrhage and congestion. In the oesophagus submucosal glands were swollen. Gizzard showed separation of mucosal layer from muscular layer and separation of muscle. Sinusoidal congestion and kupffer cell hyperplasia also observed in the liver. Myocardium of heart showed congestion and focal areas of haemorrhages. Sloughing of mucosal layer observed in trachea. Lung showed infiltration of polymorphonuclear cells in the thickened inter alveolar space. Similar lesions were described by McDougald (2003) and Al-Sadi and Hamodi (2011) in naturally occurring and experimentally induced trichomoniasis in pigeons, thus conforming our findings. Contrary to our report, Begum *et al.* (2008) did not observe microscopic lesions in the crop and proventriculus despite the presence of predominant gross lesions. According to Villanua *et al*. (2006), variation in pathologic changes was due to heterogeneity and variations in the virulence of strains involved. Presence of avirulent strains might have contributed to absence of any lesions or the lesion might be in subclinical form. The histopathological alterations in the lungs as thickening of alveolar epithelium, infiltration of polymorphonuclear cells, congestion of the blood vessels, prominent areas of haemorrhages and focal areas of necrosis were in conformation to reports of Borji *et al.* (2011). Similarly, Stoute *et al.* (2009) observed pulmonary lesion, characterized by caseonecrotic debris and infiltration of heterophils, lymphocytes and plasma cells. However, McDougald (1992) never reported lesions caused by *T. gallinae* in the digestive tract below the proventriculus. The present microscopical changes in liver are similar to those reported by Soulsby (2012) and Borji *et al.* (2011) who additionally recorded hyperemia and bile duct hyperplasia in moderately infected pigeons. Presence of focal abscesses in the liver, with an inflammatory reaction characterized by mononuclear cells and heterophils, was also reported by Begum *et al.* (2008).

CONCLUSION

Conclusively, status of *T. gallinae* infection in domestic pigeons of Assam was explored according to age and sex of birds and seasonal occurrence. Exudation of foul smelling fluid through mouth and presence of yellowish white caseated mass in the oral cavity and other parts of upper digestive tract were characteristic of trichomoniosis. Gross and histopathological study revealed ulcerative, necrotic, haemorrhagic and inflammatory lesions in different parts of affected digestive tract and liver.

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