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# Prevalence of *Trichomonas gallinae* in Domestic Birds in Assam, India

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**A B S T R A C T**

The present study was undertaken to ascertain the prevalence of *Trichomonas gallinae* inhabiting the upper digestive tract of domestic birds which included pigeon (*Columba livia domestica*), chicken (*Gallus gallus domesticus*), duck (*Anas platyrhynchos domesticus*) and quail (*Coturnix coturnix japonica*) in the state of Assam, India. A total of 1207 throat swab samples from birds (1132 live and 75 carcasses) were examined, out of which 349 birds were found positive with an overall prevalence of 28.91%. Observation on the prevalence of *T. gallinae* was done by Giemsa staining and culture. In pigeon, the prevalence was recorded as 71.12% and in chicken it was 6.25% while no *T. gallinae* was observed in duck and quail. In pigeon, prevalence was found in squab as 79.47% which was the highest. Female birds showed a prevalence rate of 75.51% while in male, it was 66.36%. In chicken prevalence rate was 6.73% in females and 6.00% in males. Season wise, highest number of cases (87.12%) in pigeon was recorded in winter and lowest in monsoon (60.58%). In chicken, *T. gallinae* infection was recorded only in two seasons; post monsoon showed slightly higher prevalence (15.49%) than winter (13.79%).

## Introduction

Birds have been domesticated by human from the ancient time to provide supplementary income to most of the landless families. Among all the domestic birds duck, chicken, geese, pigeon, quail, turkey etc. are more popular throughout the world. Within the livestock sector, poultry emerges as the most efficient sub-sector in its use of natural resources and in providing protein to supply a global growing demand. Amongst livestock, poultry sector is the smallest contributor of Greenhouse gas (GHG) emission (GLEAM 2, 2016).

A large number of ecto and endoparasites are found associated with several diseases of birds. digestive tract protozoan parasites mainly the tissue protozoa like coccidia and *Trichomonas* are known to be harmful to domestic birds in different countries including India (Jahan *et al.*, 2011; Eljadar *et al.*, 2012). *Trichomonas gallinae*, a flagellate protozoan parasite causes avian trichomoniasis in a wide range

of birds. The parasite is found mainly in the bird's anterior digestive tract and pathological lesions are confined in the crop, proventriculus, gizzard and liver and they can cause granulomatous lesions that block the oesophagic lumen, leading to death of the birds. With highly virulent isolates, a single trichomonad can cause death of pigeon within 14 days and mortality may be as high as 50% in adults (McDougald, 1992). The disease is cosmopolitan in distribution. Villanua *et al.*, (2006) studied the prevalence of trichomoniasis in common wood pigeon in Spain and found 34.2% occurrence of *T. gallinae*. Sex wise, there was no significant difference in the prevalence rate. Saikia (2016) studied the prevalence of *T. gallinae* in pigeons in Assam by throat swab examination and recorded a prevalence of 26.85%**.** Prevalence was recorded on the basis of age groups, sex and season; yellowish-white caseous lesions, characteristic of trichomoniasis were seen in the oral cavity including palate, beak, oropharynx and crop (Saikia, 2016). The present work was carried out as no detailed work on trichomoniasis has been reported from India in poultry birds except few works on pigeon.

## Materials and Methods

The study was conducted for two years w.e.f. April 2017 to March 2019 in 8 districts of Assam. Prevalence was studied according to species, age (< 30 days, young: 30 -90 days and adult >90 days) and sex of bird hosts and different seasons of the year. Regular visits were made to different households, market places, commercial farms and temple premises for collection of samples such as throat swab and carcasses found dead on the spot for laboratory examination/ evaluation.

### Physical examination of birds and sample collection for *Trichomonas gallinae*

A total of 1132 randomly selected live domestic birds (pigeon-464, chicken-304, duck-237 and quail-202) were physically examined for detection of illness with symptoms such as depression, anorexia, ruffled feather, droopiness, presence of caseous lesions on the beak, buccal cavity and diarrhoea and the observations were recorded properly. Sterilized cotton swabs (15 cm in length) soaked in normal saline solution were placed into the oropharynx and up to the crop of the birds. Entire wall of the crop was touched in a swirling motion with the swab and withdrawn and at first a smear was prepared in grease free microslide and properly labeled, wrapped with paper and brought to the laboratory for further staining and examination. After preparation of smear, the swab was cut at 1 cm and put into the culture tube containing Medium 199. Mucous material present in the swab was transferred to the media by repeated shaking of the tube.

The tubes were tightly capped and labeled properly. On reaching laboratory, the tubes were kept in a B.O.D. incubator at 37$^{o}$C anticipating the growth of *Trichomonas gallinae* if present, for their detection in wet mount preparation and stained smear microscopy. During the study period 75 bird carcasses (pigeon-52, chicken-19 and duck- 4) collected from farms, temple premises, households etc. were brought to the laboratory for detection of *T. gallinae* in the crop by similar procedure.

### Wet mount preparation

The throat swabs were squeezed thoroughly in the fluid contents of the tubes. One or 2 drops of well mixed fluid were taken on a clean glass slide pre-warmed at 37$^{o}$C, covered with a cover slip and immediately examined under low power (10X) and high power (40X) objectives of a compound microscope. The parasites if present were detected by their characteristic vigorous jerky movement (Tasca and De Carli, 2003).

### Staining of smear/permanent mount

Two to three drops of well mixed culture medium were taken on a clean glass slides and spread to air dry thoroughly. These smears were fixed in methanol for 2 minutes and stained for 30-40 minutes with diluted Giemsa's stain as per routine procedure (Tasca and Decarli, 2003). The slides

were washed thoroughly, air dried and examined under high power (40X) and oil immersion objective (100X). Similarly, the direct smears prepared in field condition during sample collection were fixed and stained as per the above procedure for examination.

## Results and Discussion

### Prevalence of *Trichomonas gallinae*

During the present study, out of 1207 birds (1132 live and 75 dead) examined for *Trichomonas gallinae*, 349 were found positive by microscopic detection (stained smears) and culture (wet mount) of throat/oropharyngeal swab with an overall prevalence of 28.91%. In pigeon, the prevalence was recorded as high as 71.12% and in chicken it was 6.25% while no *T. gallinae* was observed in duck and quail (Table -1). Statistically, the percentage prevalence of *T. gallinae* in domestic birds was highly significant (P<0.01) by Chi square analysis.

### Morphology of *Trichomonas gallinae*

Microscopic examination of Giemsa stained throat swab smears revealed the presence of single celled, oval to pyriform flagellated *Trichomonas gallinae* protozoan approximately 6.3-15.5 µm long and 4.0-8.3 µm wide (Fig 1). The four anterior flagella arising from the basal granule were about 7- 13 µm in length. The parasite was characterized by an undulating membrane due to the presence of a recurrent flagellum closely attached to the surface that extends two-thirds the length of its body. The ovoid nucleus with an approximate longitudinal diameter of 2.5- 3 µm was found close to the base of the anterior flagella.

Variation in size and shape of trophozoites were observed in the present study. These variations could be attributed to the inherent constitution of these flagellates based upon physicochemical changes in their growth environment, or due to distortions caused by the various fixatives used during preparation (Theodorides and Olson, 1965). Trophozoites of *T. gallinae* was morphologically identical to those reported by other workers (Tasca and De Carli, 2003; Melhorn *et al.,* 2009; Amin *et al.,* 2010). The parasite can assume several shapes (Stabler, 1941). The most frequently encountered forms are pyriform or pear-shaped, spherical and amoeboid (Stabler, 1941). Spherical forms appear when conditions for parasite survival become unfavorable (Stabler, 1954).

### Pigeon

Out of 464 pigeons examined for *T. gallinae*, 330 (71.12%) were found positive. Similar high prevalence were reported by several workers, viz.57% by Nematollahi *et al.,* (2012) from Iran; 59% by McKeon *et al.,* (1997) from Australia; 67.3% by Begum *et al.,* (2008) from Bangladesh. The reason for such high infection rate might be mainly due to transmission of the parasite through feeding of crop milk to the pigeon squabs by the adult mother which can remain as constant source of infection for their young.

However, contrary to the present finding, lower infection rate of trichomoniasis in domestic and wild pigeons was also reported by many other workers, viz. Saleem *et al.,* (2008) from Lahore, Pakistan (43%), Qiu *et al.,* (2012) from China (33.9%), Bahrami *et al.,* (2012) from Iran (26.8%). Saikia (2016) earlier reported a prevalence of 26.85% in domestic pigeons of Assam and it was much lower than that observed in the present study. The wide variation in the prevalence rate might be due o the method of examination which involved only throat swab screening by microscopic examination in the earlier case.

Gross lesions such as yellowish-white caseous lesions, etc. characteristic of trichomoniasis were seen in the oral cavity including palate, beak, oropharynx and crop in clinical cases mainly in pigeons (Fig 2). Associated clinical symptoms such as swollen beak, drooling of saliva, puffed up appearance of mouth, pendulous crop, ruffled feathers were also observed in few cases of pigeon.

Several workers like Abd El-Rahman *et al.,* (2008) from Qualiobia governorate; Sansano-Maestre *et al.,* (2009) from Spain; Al- Sadi and Hamodi (2011) from Iraq and Borji *et al.,* (2011) from Iran also detected clinical symptoms.

In our present investigation, both pathogenic and non-pathogenic strains were observed showing varied pathologic lesions in the upper digestive tract from a mild inflammation of the mucosa to caseous areas that blocked the oesophageal lumen, similar to the findings of Stabler (1954). Stabler (1948) reported that 80– 90% of adult pigeons were infected without showing any clinical signs of the disease which may be due to the birds becoming immunized as a result of exposure to an avirulent strain of the parasite, enabling them to act as a constant source of infection for their progenies.

## Chicken

In chicken, out of 304 throat swabs examined, 19 birds were positive either by direct smear or culture method, with 6.25% prevalence. Chicken positive for infection were apparently healthy exhibiting mild lesions restricted to oral cavity only. There is not enough literature to compare the prevalence of natural cases of trichomoniasis recorded in the present study. However, Levine and Brandly (1940) investigated chickens in Illinois and reported *T. gallinae* causing disease of upper digestive tract. Willoughby *et al.,* (1995) diagnosed two cases of esophageal trichomoniasis due to *T. gallinae* in backyard chicken flocks ranging from 12 weeks to 1 year of age with clinical signs of watery eyes, open mouthed breathing, drooling of saliva from the mouth and nostrils etc. at the California Veterinary Diagnostic Laboratory System and the organisms were readily demonstrated on wet smears.

## Duck and Quail

Not a single positive case of *T. gallinae* could be recorded in duck and quail either by direct smear examination or *in vitro* culture (wet mount) method during the present study. The result indicated that duck and quail generally do not harbor infection as they may not be the natural hosts of *T. gallinae* and are resistant to the parasite. Tsai *et al.,* (1997) described an outbreak of trichomonosis due to *Tetratrichomonas anatis* in farmed ducks with respiratory and intestinal presentation. The clinical signs of the respiratory form resembled *Trichomonas gallinae* infections affecting the upper respiratory tract, infraorbital sinuses and nasal cavity with tracheitis, mucofibrino-purulent and catarrhal rhinitis.

## Prevalence of *Trichomonas gallinae* infection according to age group

The results of age-wise prevalence are shown in Table-2. In pigeon, out of 190 samples examined from squab, 151 were found positive with prevalence of 79.47% which was the highest. In young bird, 88 out of 144 samples were positive (61.11%) and in adults, 91 were positive for *T. gallinae* out of 130 samples examined, the prevalence being 70.00%. Statistically, the percentage prevalence of *T. gallinae* in different age group was highly significant ($P<0.01$) by Chi square analysis. The present finding is in accordance with other workers (Abdel-Motelib *et al.,* 1997; Butcher, 2003; McDougald, 2003). Qiu *et al.,* (2012) examined freshly prepared wet mounts of 319 domestic pigeons in Southern China and reported significant difference in the prevalence of *T. gallinae* in pigeons in different ages with breeding birds having a lower prevalence compared to adolescent birds and nestlings. Similarly, Saikia (2016) reported highest prevalence of trichomoniasis in squab (56.25%) followed by young (22.38%) and lowest in adult pigeon (10.90%) of Assam by throat swab examination. The main route of transmission of the organism is feeding squabs “crop milk” by adult pigeons, a mixture of the secretion from the crop glands and regurgitated food, thereby infecting the naive squabs with organisms present in their mouths and crops. Possibility of getting infection in squab might be due to weak immune system. Carrier pigeons are known to transmit trichomonads to their young during feeding (Butcher, 2003).

**Table.1** Prevalence of *Trichomonas gallinae* in domestic birds

| Bird/Host | No. of birds examined | No. of birds positive | Prevalence % | Chi square value | P- value |
|---|---|---|---|---|---|
| **Pigeon** | 464 | 330 | 71.12 | **311.75**** | **< 0.001** |
| **Chicken** | 304 | 19 | 6.25 | | |
| **Duck** | 237 | 0 | 0.00 | | |
| **Quail** | 202 | 0 | 0.00 | | |
| **Total** | **1207** | **349** | **28.91** | | |

** Highly Significant (P<0.01).

**Table.2** Age wise prevalence of *Trichomonas gallinae* in domestic birds

| Species of bird examined | Age group | No. of sample examined | No. of sample positive | % prevalence | Chi square value | P-value |
|---|---|---|---|---|---|---|
| **Pigeon** | Squab | 190 | 151 | 79.47 | **13.56**** | **0.001** |
| | Young | 144 | 88 | 61.11 | | |
| | Adult | 130 | 91 | 70.00 | | |
| **Total** | | **464** | **330** | **71.12** | | |
| **Chicken** | Chick | 89 | 4 | 4.49 | **1.20**$^{NS}$ | **0.55** |
| | Young | 125 | 10 | 8.00 | | |
| | Adult | 90 | 5 | 5.55 | | |
| **Total** | | **304** | **19** | **6.25** | | |
| **Duck** | Duckling | 90 | 0 | 0 | | |
| | Young | 81 | 0 | 0 | | |
| | Adult | 66 | 0 | 0 | | |
| **Total** | | **237** | **0** | **0** | | |
| **Quail** | Chick | 47 | 0 | 0 | | |
| | Young | 70 | 0 | 0 | | |
| | Adult | 85 | 0 | 0 | | |
| **Total** | | **202** | **0** | **0** | | |

**Highly significant (P<0.01), $^{NS}$Non Significant (P>0.05).

**Table.3** Sex wise prevalence of *Trichomonas gallinae* in domestic birds

| | Pigeon | | Chicken | | Duck | | Quail | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female |
| **No. of sample examined** | 223 | 241 | 200 | 104 | 140 | 97 | 122 | 80 |
| **No. of positive sample** | 148 | 182 | 12 | 7 | 0 | 0 | 0 | 0 |
| **% prevalence** | **66.36** | **75.51** | **6.00** | **6.73** | **0** | **0** | **0** | **0** |
| **Chi square value** | **4.722*** | | **0.062**$^{NS}$ | | | | | |
| **P- value** | **0.029** | | **0.803** | | | | | |

* Significant P (<0.05), $^{NS}$ Non significant (P>0.05)

**Table.4** Seasonal prevalence of *Trichomonas gallinae*

| Month/ season | No. of Samples screened for *Trichomonas gallinae* in different species of birds | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Pigeon** | **Positive %** | Chi square value, P- value | **Chicken** | **Positive %** | Chi square value, P- value | **Duck** | **Positive %** | **Quail** | **Positive %** |
| Pre monsoon (March, April, May) | 130 (89) | 68.46 | **20.61**,** | 75 (0) | 0% | **0.07,** | 60 (0) | 0% | 55 (0) | 0% |
| Monsoon (June, July, August, September) | 137 (83) | 60.58 | | 100 (0) | 0% | | 73 (0) | 0% | 44(0) | 0% |
| Post monsoon (October, November) | 96 (70) | 72.91 | **0.0001** | 71 (11) | 15.49% | **0.79$^{NS}$** | 42(0) | 0% | 52(0) | 0% |
| Winter (December, January, February) | 101(88) | 87.12 | | 58 (8) | 13.79% | | 62(0) | 0% | 51(0) | 0% |
| **Total** | **464(330)** | **71.12** | | **304 (19)** | **6.25%** | | **237(0)** | **0%** | **202(0)** | **0%** |

**Highly significant, (P<0.01) $^{NS}$Non-significant (P> 0.05)

**Fig.1** Microscopic view of Trophozoites of *Trichomonas gallinae* in throat swab smear (Giemsa stained), (1000X)

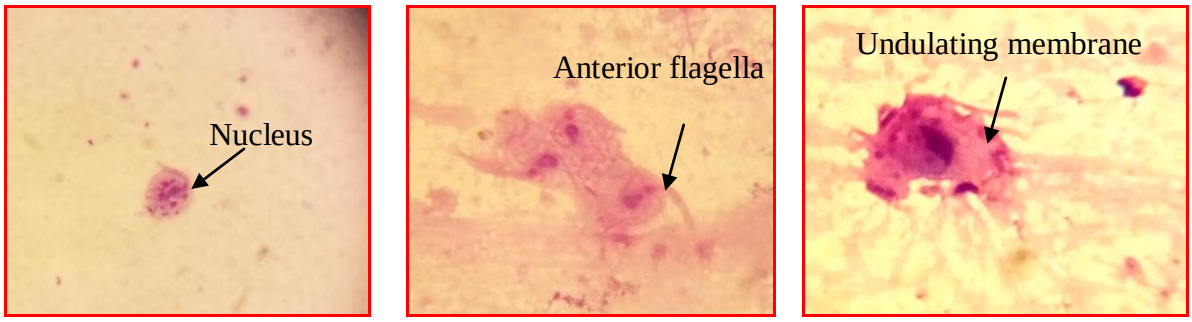
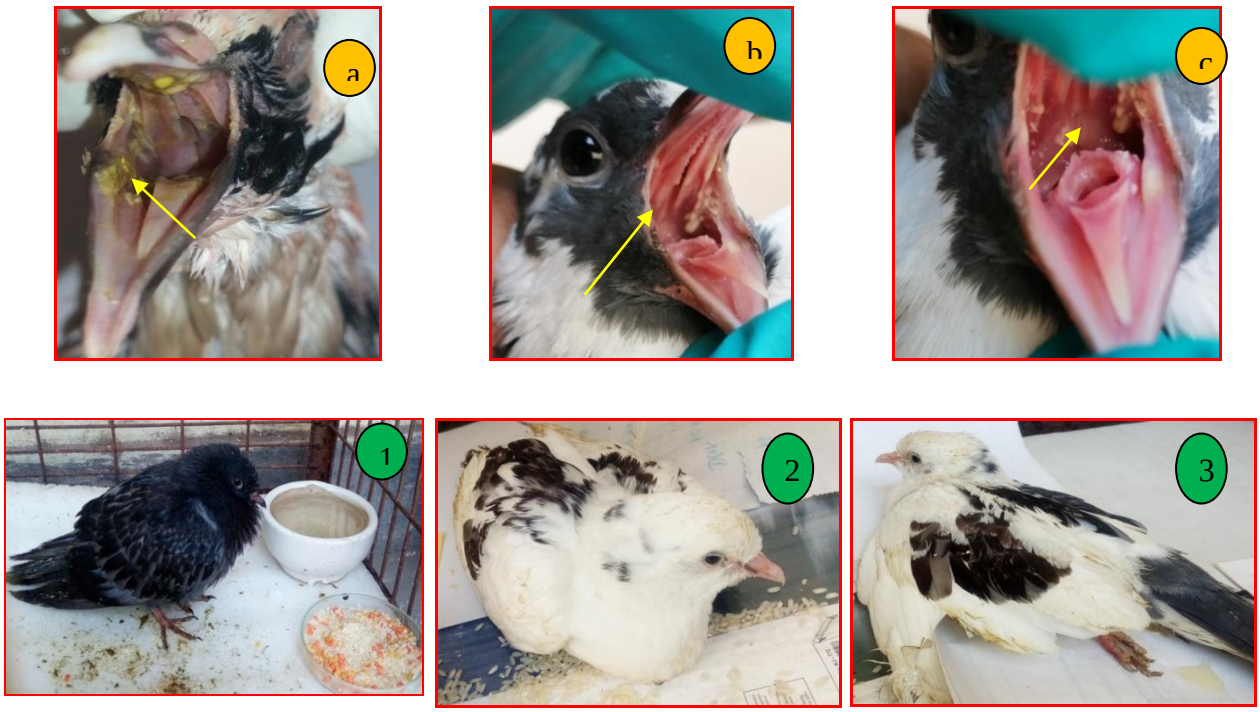



**Fig.2** Oral Necrotic lesions (a-c) and Clinical symptoms of trichomoniasis in pigeon (1-3)




In the present study, trichomoniasis was reported in apparently healthy pigeons. This finding may indicate that trichomoniasis could occur in the absence of secondary disease. In contrast to the present findings, Begum *et al.*, (2008) reported highest prevalence of *T. gallinae* in adult (75%) followed by squabs (72.1%) and young (64.7%). In chicken, out of 89 throat/oropharyngeal samples examined in chicks, 4 nos. were found positive with prevalence of 4.49%. In young age group, 10 out of 125 samples were positive for the organism (8.00%) as the highest and in adult it was lowest with 2.96% (4/135). Statistically, the percentage prevalence of *T. gallinae* in different age group was non-significant (P>0.05).

However, Levine and Brandly (1940) and Willoughby *et al.*, (1995) reported cases of upper digestive tract and esophageal trichomoniasis respectively in young and adult chickens.

Examination of throat swab samples consisting of 90 nos. from ducklings, 81 nos. from young age group and 66 nos. from adult ducks showed no positive results for *T. gallinae*. However, Tsai *et al.,* (1997) described an outbreak of respiratory and intestinal trichomonosis in farmed adult ducks.

In quails, 47 throat/oropharyngeal samples from chicks, 70 nos. from young and 85 nos. from adult age groups were examined and all samples were found negative for *T. gallinae*. There is paucity of literature to compare the prevalence of *T. gallinae* in quail.

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

The prevalence of *T. gallinae* infection according to sex of bird is presented in Table-3. Out of 464 throat swab samples screened in pigeons, 241 samples from female birds resulted in a prevalence rate of 75.51% while in male, the corresponding value was 66.36%.

Comparatively higher prevalence was observed in female than in male pigeon and this variation was also statistically found significant (P<0.05) by Chi square analysis. The present finding agrees to the report of Begum *et al.,* (2008); Abed *et al.,* (2014) and Saikia (2016).

Contradictively, Al- Sadi and Hamodi (2011) recorded higher infection in male than in female pigeons while Villanua *et al.,* (2006) in their study conducted in common wood pigeon in Spain found no significant difference in prevalence of *T. gallinae* sex wise. The cause of higher prevalence of *T. gallinae* infection in females could not be justified but according to Lloyd (1983) it could be assumed that female sex hormones might play a role making them more susceptible to any infection.

In chicken, a total of 304 throat swab samples were examined consisting of 104 from female birds and 200 from males which resulted a prevalence of 6.73% and 6.00% in females and male birds respectively. Slightly higher prevalence was observed in female than in male birds and this variation was statistically found non-significant (P>0.05) by Chi square analysis.

Examination of 237 throat samples from duck (140 female and 97 male) gave no positive results for *T. gallinae*.

In quail, 122 nos. of samples from male and 80 nos. of samples from female were examined and no positive results were seen among the two sexes.

**Seasonal prevalence of *Trichomonas gallinae* infection in birds**

The entire period of study was divided into four seasons to put on record the effect of season on the prevalence of *Trichomonas gallinae* infection in domestic birds as shown in Table-4. Clinical and subclinical illness in pigeon due to *T. gallinae* was recorded throughout the year. Season wise, the parasite positivity in pigeon ranged from 60.58% to 87.12% and in the case of chicken 13.79% to 15.49%.

Highest number of cases (87.12%) in pigeon was recorded in winter followed by post monsoon (72.91%), pre monsoon (68.46%) and monsoon (60.58%). In chicken *T. gallinae* infection was recorded only in two seasons, post monsoon showed slightly higher prevalence (15.49%) than winter (13.79%). The variation of prevalence percentage of *T. gallinae* in different seasons was found statistically highly significant in pigeon (P<0.01) while non significant (P>0.05) in chicken by Chi-square analysis.

The highest prevalence of *T. gallinae* in domestic pigeons in winter season followed by post monsoon and pre monsoon as observed in the present study agrees to the earlier report made by Saikia (2016) while working in domestic pigeons of Assam. However, Begum *at al*. (2008) recorded highest prevalence in monsoon (69.8%) followed by winter (69.3%) and summer seasons (48.4%) which

contradicts our findings. The seasonal difference might be due to climatic change, sampling size of the birds etc. Literature is scarce to compare with the present report of seasonal prevalence of *T. gallinae* infection in chicken.

The present study recorded overall prevalence of trichomoniasis as 28.91%. Only pigeon and chicken were found to be positive by throat swab examination while no *T. gallinae* was observed in duck and quail. Females showed slightly higher prevalence than male birds and Season wise, prevalence in pigeon was recorded throughout the year.

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