



International Journal of Biology Sciences

ISSN Print: 2664-9926
ISSN Online: 2664-9934
NAAS Rating (2026): 4.82
IJBS 2026; 8(4): 11-18
www.biologyjournal.net
Received: 21-01-2026
Accepted: 23-02-2026

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Cross transmission study and pathogenesis of *Trichomonas gallinae* in different hosts in Assam, India

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DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i4a.613>

Abstract

To determine the virulent nature of the *Trichomonas gallinae* and its transmissibility to different hosts, experiment was performed with or without immunosuppressive drugs in chicken, duck, quails and mice taking pigeon as its natural host. Pigeon strain of *T. gallinae* orally inoculated at concentration of 4×10^4 trophozoites in birds and intra peritoneal inoculation in mice revealed presence of parasites in all bird species while mice developed abscess which is an indicator of *T. gallinae* infection.

Keywords: *Trichomonas gallinae*, transmission, pigeon, chicken, duck, quail, mouse

Introduction

Trichomonas gallinae, the causative agent of Avian trichomoniasis, is a flagellate protozoan parasite under class Zoomastigophorea and order Trichomonadida. In natural condition, this parasite occur mainly in the columbiformes birds (pigeon and mourning dove) although several other birds including wild carnivores (raptors) and domestic birds (chicken, quail, pheasants) may be infected (Girard *et al.*, 2014) [8]. The disease, often known as canker, has significant health and economic impact on poultry industry, especially in pigeon and game birds rearing and breeding (Stockdale *et al.*, 2015) [21] and is considered as an emerging and potentially fatal disease of birds leading to decline of avian populations (Robinson *et al.*, 2010; Girard *et al.*, 2014) [16, 8]. Although, the main site of predilection of *T. gallinae* is mainly the upper digestive tract, the respiratory system and other visceral organs may also be involved in the pathogenic process (Borji *et al.*, 2011) [2]. Surviving birds remain as carrier and become a source of infection for others.

Transmission of *T. gallinae* by experimental means has been performed in avian and mammalian hosts by many workers. In columbiform birds, experimental transmission by oral intubation of cultured or fresh parasite suspensions has been used and many of the workers reported simple cross-transmission experiments to establish whether or not *T. gallinae* would produce disease. (Cauthen, 1936; Powell and Hollander, 1982) [3, 15]. Stabler (1948) [18] also observed that transmission of highly pathogenic strain of *T. gallinae* obtained from healthy carriers when inoculated in pigeons induced fatal caseo-necrotic lesions that lasted only for two to four days. According to Honiberg (1961) [9], *Mus musculus* was the most frequently used mammal for studying *T. gallinae* pathogenicity by subcutaneous inoculation. The infected mice developed abscesses or pustules rather than pronounced caseous nodules as in birds.

Materials and Methods

Cross transmission study of *Trichomonas gallinae* was conducted by experimental inoculation of the parasite (isolated from pigeon and cultured *in vitro*) into chicken, duck, quail and mice taking pigeon as the natural host of the parasite. All the birds and mice were reared species wise in wire netted iron cages and supplied with feed and water *ad libitum* and maintained as per the guidelines of the Animal Ethics Committee, College of Veterinary Science, Assam Veterinary and Fishery University, Assam. Throat swabs of birds and mice were examined under microscope by wet mount to ascertain *T. gallinae* status.

Grouping of the birds and mice

Twelve birds from each species (pigeon, chicken, duck and quail) were put into three groups (I, II, III) each with four birds (Fig 1, Table 1). Group I birds were considered as healthy control; group II were healthy without immunosuppression but experimentally inoculated (orally) with 4×10^4 trophozoites of *T. gallinae*; group III birds were both immunosuppressed and inoculated with *T. gallinae*. In

the case of mice experimentation, 9 mice were divided into three equal groups (I, II, III). However, *T. gallinae* was inoculated peritoneally. Immunosuppression was done with injection of dexamethasone sodium as per experimental design @ 0.2 ml I/M in a single dose. Oral inoculation of *T. gallinae* was also done with 4×10^4 trophozoites.

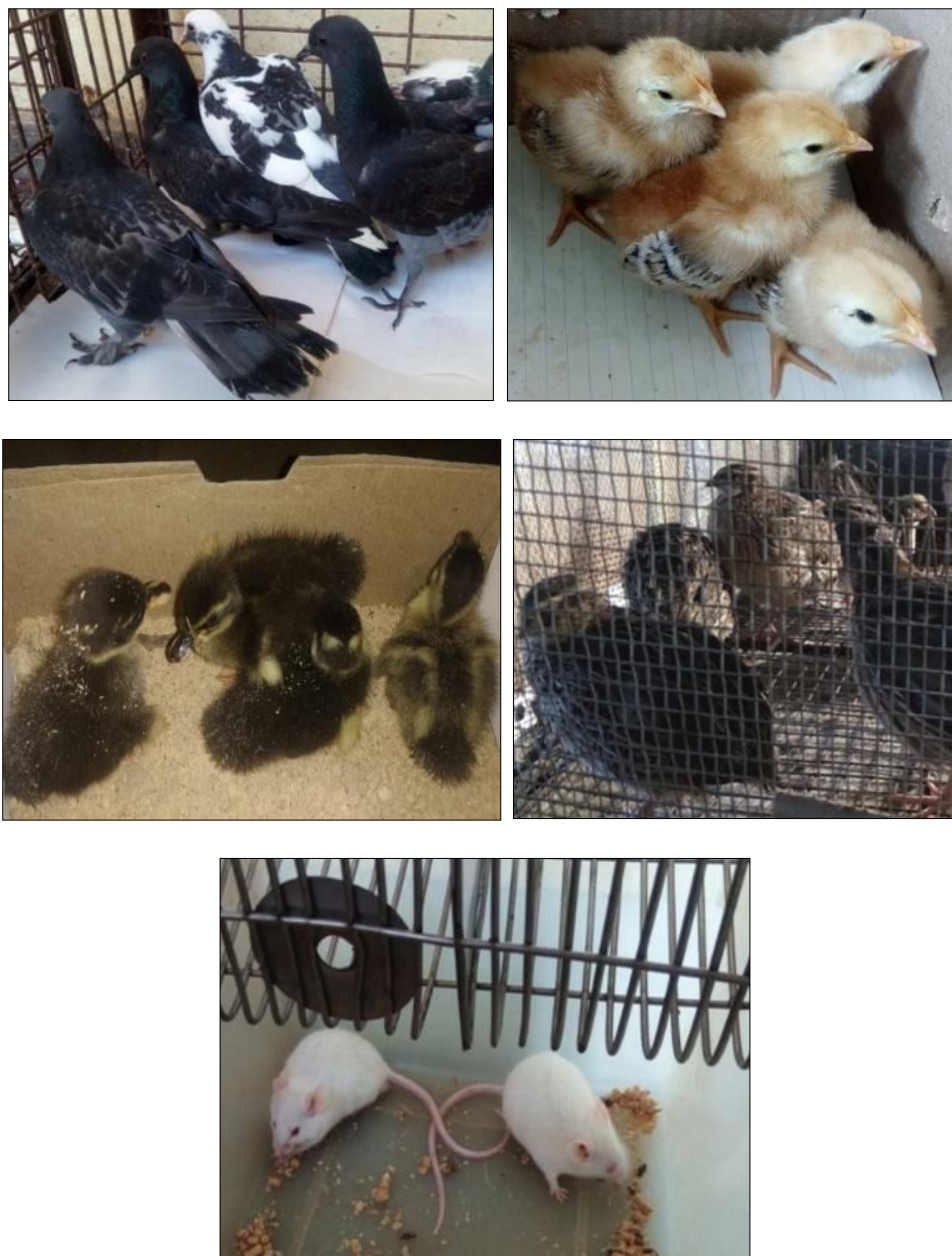


Fig 1: Grouping of birds/mice for transmissibility studies

Monitoring of infection

Experimental birds and mice of each group were kept under careful observation up to 15 days for appearance of clinical symptoms and mortality. Throat swabs were also examined on every alternate day from day 3 for detection of parasites and grading was done as very high (+++), high (+++), moderate (++), mild (+) and negative (-). Experimentally inoculated surviving birds were sacrificed after 15 days of observation for parasitological and pathological study.

Results and Discussion

Cross transmissibility studies in pigeon

Host reaction due to experimental inoculation of *T. gallinae* is presented in Table 2. The birds of group I neither showed any symptoms of illness nor parasites were detected on throat swab examination.

The group II birds developed clinical symptoms from 3rd day post inoculation such as drowsiness, decrease in appetite and from 5th day onwards exhibited ruffled feathers (Fig 2 i), slight lesions in oral cavity and from 9th day, the birds showed droopiness, and on 11th day were in cachectic condition with heavy lesions in oral cavity, around the throat and palate (Fig 3) Parasites could be detected on throat swab examination from 5th day of infection while high concentrations were seen

from 11th- 15th days post inoculation. No mortality was seen in group II birds and on 15th day, whitish fluid drooled out through beak along with necrotic lesions in the oral cavity.

The group III birds showed ruffled feather, drowsiness, decreased appetite on 3rd day post inoculation and high concentration of parasites (++++) on throat swab examination. On 7th day, moderate lesions were seen in oral cavity and on 9th day, the birds had pendulous crop (Fig 2 ii), lesion in eye, diarrhoea, inability to feed or drink water. On 11th day onwards severe lesions were grossly seen in the oral cavity, palate, throat, inability to move (Fig 2 iii), complete stoppage of feeding and drinking, with very high concentration of parasites (+++). On 13th day of infection, 3 birds died naturally due to lesions blocking the oropharyngeal region. The remaining bird was sacrificed on 15th day.

Transmission of *T. gallinae* by experimental means has been performed earlier in avian and mammalian hosts by different workers. In columbiform birds, experimental transmission by oral intubation of cultured or fresh parasite suspensions was used (Cauthen, 1936; Conti *et al.*, 1985; Powell and Hollander, 1982; Stabler and Kihara, 1954)^[3,4,15,20]. Many of the previous papers reported simple cross-transmission experiments to establish whether or not *T. gallinae* would produce disease. Powell and Hollander (1982)^[15] transmitted *T. gallinae* from pigeons to ring doves. Stabler and Herman (1951)^[19] also transmitted *T. gallinae* from mourning doves

to domestic pigeons. Intrahepatic, intramuscular, intraperitoneal and subcutaneous injections of *T. gallinae* have been used to produce trichomonad pathogenicity.

In line with the present observations, Neimanis *et al.* (2010)^[14] also reported similar clinical signs such as lethargy; gaping beaks which may be covered in food remains; laboured breathing; drooling; closed eyes; matted and wet feathers around the beak; head and chest and dishevelled feathers held away from the body exhibited by birds. Mohamed *et al.* (2009)^[13] reported that natural and experimental affected squabs with trichomoniasis showed depression, off-food and dyspnea, in addition to offensive-odor-fluid, drooling from the opened peak, due to lesions in the oral cavity. Moreover, a large caseated mass was present in the buccal cavity. Sometimes greenish watery diarrhea was noticed. Similar signs along with occlusion of the crop with caseated materials were also reported by Abd-ElMotelib and Galal (1993)^[1]. It was reported that when *Trichomonas*-free, non-immune pigeons were infected with a known avirulent *T. gallinae* isolate, they did not elicit an immune response or develop any lesion even after being inoculated with 1×10^6 organisms. In contrast, when infected with a known virulent strain, *Trichomonas*-free, non-immune pigeons died within six to nine days following inoculation of a single trichomonad.



(i) Ruffled feather



(ii) Pendulous crop



(iii) Unable to move

Fig 2: Clinical symptoms of trichomoniasis in pigeon (i-iii)





Fig 3: Oral necrotic lesions in pigeons

Cross transmissibility studies in chicken

Host reaction following experimental inoculation of *T. gallinae* in chicken is presented in Table 3.

The birds of group I neither showed any symptoms of illness nor parasites were detected on throat swab examination. The group II birds did not show any clinical symptoms till 6th day of post infection but parasites were detected on day 7. Symptoms like weakness, reduced feed and water intake etc. were observed from 9th day onwards. On 11th day of infection, the birds showed droopiness, diarrhoea and mild parasites were detected (+). No mortality was recorded till 15 day and all the birds were sacrificed.

Group III birds had diarrhoea, became drowsy with low parasites on throat swab examination from 3th day onwards. Droopiness, anorexia, severe diarrhoea with high concentration of parasites (+++) were seen from 7th day till 15th day of observation but no mortality was seen. On 15th day of infection, birds were sacrificed for detailed pathological studies.

Chickens and turkeys have been orally intubated with *T. gallinae* from pigeons (Levine *et al.*, 1941; Levine and Brandly, 1939)^[10,11]. Gabaldon and Andrews (1935)^[7] also used chickens for experimental transmission. Levine (1985)^[12] reported that infections in chickens are rare. There is paucity of literature to compare the pathogenesis and pathology of *T. gallinae* infection in chicken as observed in our present study. However, Sasikala *et al.* (2017)^[17] reported only natural case of Trichomoniasis in 2 months old Desi chicken in Orathanadu and finding *Trichomonas* sp. trophozoites by cytological examination of the scrapings from crop mucosa.

Cross transmissibility studies in duck

Host reaction following experimental inoculation of *T. gallinae* in duck is presented in Table 4.

The birds of group I neither showed any symptoms of illness nor parasites were detected on throat swab examination. From 5th day onwards, group II birds showed diarrhoea, decreased appetite and absence of gross oral lesions. Very less parasites were detected (+) in throat swab examination from 13th day onwards. No mortality was noticed in this group. Group III birds had diarrhoea, decreased appetite, moderate concentration of parasite (++) from 3rd day onwards and gradually becoming high (+++) till 15th day. On 11th day,

one bird died naturally of infection while the remaining 3 birds in cachectic condition were sacrificed.

There is not much literature available to compare the experimental design, transmission, pathogenesis and pathology of *T. gallinae* infection in duck as carried out in the present study. However, Tsai *et al.* (1997)^[22] recorded natural outbreak of trichomonosis due to *T. gallinae* in farmed ducks showing signs and symptoms of respiratory and intestinal form.

Cross transmissibility studies in quail

Host reaction following experimental inoculation of *T. gallinae* in quail is presented in Table 5.

In birds of group I (uninfected control) no clinical symptoms/lesions were seen and parasites were also not detected on throat swab examination. In group II birds no gross lesion or symptoms was noticed till 15th day and all birds were sacrificed. In group III birds till 7th day of infection, no alteration was observed. On 9th day birds having diarrhoea gradually showed signs of weakness and ruffled feathers. Parasite were detected from 13th day onwards (+) but no mortality was seen and all birds were sacrificed on 15th day of infection.

In line with our present findings, Levine *et al.* (1941)^[11] reported the results of feeding experiments on the transmission of *Trichomonas* to Bobwhite quails. No lesions were found in the trachea, liver or other abdominal organs of any of the quail and trichomonads were not found in the intestinal tract posterior to the gizzard thus agreeing to our report. Numerous protozoa were demonstrable in the necrotic areas and in the yellowish fluid seen in the crop and proventriculus of some of the infected quails.

Cross transmissibility studies in mice

Host reaction following experimental inoculation of *T. gallinae* in mice is presented in Table 6.

Group I mice (uninfected control) were normal in health and activity. In group II mice no mortality was noticed till 15 days of experiment and all mice were sacrificed. In group III, all mice appeared normal and apparently healthy till 7th day. Gradually they started showing symptoms of weakness, decreased appetite, inability to move, rough body hair coat and one mouse died on day 13th while the remaining 2 mice were sacrificed on 15th day of infection.

Several workers (Frost and McLure, 1961; Frost and Honigberg, 1962) ^[5,6] carried out experimental study of trichomonads in mice. Honigberg (1961) ^[9] opined that the most frequently used mammal for studying trichomonad pathogenicity was *Mus musculus*. He investigated the

pathogenicity of *T. gallinae* by conducting “Mouse Assay” technique and compared the mean volumes of lesions on the 2nd day to the 14th day produced by various strains of trichomonads after subcutaneous inoculation of dose in pure bred mice.

Table 1: Experimental design of cross transmission study of *T. gallinae* in birds and mice

Experimental host/species	Group	No. of birds/mice	Immuno-suppression*	Experimental inoculation**	Remarks
Pigeon (6weeks old)	I	4	-	-	Healthy control
	II	4	-	+	Healthy inoculated
	III	4	1+	+	Immunosuppression and inoculated
Chicken (2weeks old)	I	4	-	-	HC
	II	4	-	+	HI
	III	4	1+	+	II
Duck (3weeks old)	I	4	-	-	HC
	II	4	-	+	HI
	III	4	1+	+	II
Quail (2weeks old)	I	4	-	-	HC
	II	4	-	+	HI
	III	4	1+	+	II
Mice (2weeks old)	I	3	-	-	HC
	II	3	-	+	HI
	III	3	1+	+	II

N.B: * Dexamethasone 0.2ml I/M **4X10⁴ *T. gallinae* per inoculation Observation period=15 days

Table 2: Host reaction after experimental inoculation of *T. gallinae* in pigeon

Dose of <i>T. gallinae</i> inoculum	Day of experiment	Host reaction in pigeons					
		Group I (control group)		Group II (Infected but not immunosuppressed)		Group III (Infected and immunosuppressed)	
		Symptoms observed	Throat swab examination	Symptoms observed	Throat swab examination	Symptoms observed	Throat swab examination
	Day 1	Normal	Parasite -ve	Normal	Parasite -ve	Normal	Parasite -ve
	Day 3	Normal	Parasite -ve	Drowsiness and decrease in appetite	Parasite -ve	Ruffled feather, drowsiness, decrease in appetite	Parasite +ve (+++)
	Day 5	Normal	Parasite -ve	Weakness, Anorexia	Parasite +ve (+)	Cachectic condition	Parasite +ve (+++)
4x10 ⁴	Day 7	Normal	Parasite -ve	Ruffled feathers, drowsiness, slight lesion in oral cavity, reduced feed & water intake	Parasite +ve (+)	Moderate lesions seen in oral cavity	Parasite +ve (+++)
	Day 9	Normal	Parasite -ve	Droopiness, activity of the birds reduced	Parasite +ve (++)	Pendulous crop was seen in one bird, eye lesion also seen, loss of body weight, diarrhoea, inability to feed or drink water	Parasite +ve (+++)
	Day 11	Normal	Parasite -ve	Cachectic condition	Parasite +ve (+++)	Severe lesions in the oral cavity, palate, throat region, birds unable to move, complete stoppage of feeding and drinking	Parasite +ve (++++)
	Day 13	Normal	Parasite -ve	Lesions seen in oral cavity, around the throat & palate	Parasite +ve (+++)	3 birds died naturally with lesions blocking oropharyngeal region	Parasite +ve (++++)
	Day 15	Normal	Parasite -ve	Whitish fluid coming through beak, necrotic lesion in oral cavity; all birds were sacrificed	Parasite +ve (+++)	Severe lesions in oropharynx; remaining bird was sacrificed	Parasite +ve (++++)

N.B: Oral inoculation of *T. gallinae* in Group II and III on day 1; Immunosuppressive injection to Group III one day ahead of oral inoculation

Table 3: Host reaction due to experimental inoculation with *T. gallinae* (pigeon strain) in chicken

Dose of <i>T. gallinae</i> inoculum	Day of experiment	Host reaction in chickens					
		Group I (Control group)		Group II (Infected but not immunosuppressed)		Group III (Infected and immunosuppressed)	
		Symptoms observed	Throat swab examination	Symptoms observed	Throat swab examination	Symptoms observed	Throat swab examination
4x10 ⁴	Day 1	Normal	Parasite -ve	Normal	Parasite -ve	Normal	Parasite -ve
	Day 3	Normal	Parasite -ve	No alteration in general health condition	Parasite -ve	Diarrhoea, drowsiness	Parasite +ve (+)
	Day 5	Normal	Parasite -ve	Normal	Parasite -ve	Reduction in feed consumption	Parasite +ve (++)
	Day 7	Normal	Parasite -ve	Normal	Parasite +ve (+)	Weakness, droopiness, anorexia and diarrhoea	Parasite +ve (+++)
	Day 9	Normal	Parasite -ve	Slight weakness, reduced feed and water intake	Parasite +ve (+)	Ruffled feather, severe weakness	Parasite +ve (+++)
	Day 11	Normal	Parasite -ve	Weakness of the affected birds with droopiness	Parasite +ve (+)	No mortality was observed, anorexic	Parasite +ve (+++)
	Day 13	Normal	Parasite -ve	No mortality, slight diarrhoea	Parasite +ve (+)	Diarrhoea	Parasite +ve (+++)
	Day 15	Normal	Parasite -ve	Whitish diarrhoea	Parasite +ve (+)	Very weak	Parasite +ve (+++)

N.B: Oral inoculation of *T. gallinae* in Group II and III on day 1

Table 4: Host reaction due to experimental inoculation with *T. gallinae* (pigeon strain) in duck

Dose of <i>T. gallinae</i> inoculum	Day of experiment	Host reaction in duck					
		Group I (Control group)		Group II (Infected but not immunosuppressed)		Group III (Infected and immunosuppressed)	
		Symptoms observed	Throat swab examination	Symptoms observed	Throat swab examination	Symptoms observed	Throat swab examination
4x10 ⁴	Day 1	Normal health	Parasite -ve	Normal and free from any parasitic infection, birds appeared healthy	Parasite -ve	Normal health	Parasite -ve
	Day 3	Normal	Parasite -ve	No alteration in general health; birds were active	Parasite -ve	Diarrhoea and decrease in appetite	Parasite +ve (++)
	Day 5	Normal	Parasite -ve	Birds showed symptoms of diarrhoea	Parasite -ve	No lesion seen in oral cavity, birds were less active with decrease in appetite	Parasite +ve (+++)
	Day 7	Normal	Parasite -ve	No lesion in oral cavity, activity normal	Parasite -ve	Affected birds showed diarrhea, reduction in intake of feed and water	Parasite +ve (+++)
	Day 9	Normal	Parasite -ve	Decrease in appetite of birds	Parasite -ve	Birds were sick with ruffled feathers and less intake of feed	Parasite +ve (+++)
	Day 11	Normal	Parasite -ve	Feeding reduced and no lesions were seen in oral cavity	Parasite -ve	One bird died naturally	Parasite +ve (+++)
	Day 13	Normal	Parasite -ve	Diarrhoea was seen	Parasite +ve (+)	Anorexia and severe weakness	Parasite +ve (+++)
	Day 15	Normal	Parasite -ve	Weakness and sacrificed after 15 days	Parasite +ve (++)	Cachectic condition and sacrificed after 15 days	Parasite +ve (+++)

N.B: Oral inoculation of *T. gallinae* in Group II and III on day 1

Table 5: Host reaction due to experimental inoculation with *T. gallinae* (pigeon strain) in quail

Dose of <i>T. gallinae</i> inoculum	Day of experiment	Host reaction in quail					
		Group I (Control group)		Group II (Infected but not immunosuppressed)		Group III (Infected and immunosuppressed)	
		Symptoms observed	Throat swab examination	Symptoms observed	Throat swab examination	Symptoms observed	Throat swab examination
	Day 1	Normal health	Parasite -ve	Normal health	Parasite -ve	Normal health	Parasite -ve
	Day 3	Normal	Parasite -ve	Normal	Parasite -ve	Normal appetite of the birds	Parasite -ve
	Day 5	Normal	Parasite -ve	Normal	Parasite -ve	No lesion seen	Parasite -ve

4x10 ⁴	Day 7	Normal	Parasite -ve	Normal	Parasite -ve	Normal	Parasite -ve
	Day 9	Normal	Parasite -ve	Normal	Parasite -ve	Diarrhoea	Parasite -ve
	Day 11	Normal	Parasite -ve	Normal	Parasite -ve	Decrease appetite	Parasite -ve
	Day 13	Normal	Parasite -ve	Normal	Parasite -ve	Weakness and ruffle feather	Parasite +ve (+)
	Day 15	Normal	Parasite -ve	Normal, sacrificed	Parasite -ve	Sacrificed	Parasite +ve (++)

N.B: Oral inoculation of *T. gallinae* in Group II and III on day

Table 6: Host reaction due to experimental inoculation with *T. gallinae* (pigeon strain) in mice

Host reaction in mice				
Dose of <i>T. gallinae</i> inoculum	Day of experiment	Group I (Control group)	Group II (Infected but not immunosuppressed)	Group III (Infected and immunosuppressed)
		Symptoms observed	Symptoms observed	Symptoms observed
4x10 ⁴	Day 1	Normal health	Normal health	Normal health and appetite
	Day 3	Normal	Normal	Normal
	Day 5	Normal	Normal	Normal
	Day 7	Normal	Normal	Normal
	Day 9	Normal	Normal	Slight weakness
	Day 11	Normal	Normal	Less intake of food and water
	Day 13	Normal	Normal	Unable to move and weakness, rough hair coat, one mouse died naturally
	Day 15	Normal	Normal condition and all the mice were sacrificed	Remaining 2 mice sacrificed

N.B: Intra peritoneal inoculation of *T. gallinae* in Group II and III on day 1

Conclusion

From the present study it could be concluded that detailed study on cross transmission and pathogenesis of *T. gallinae* isolated from pigeon into chicken, duck, quail and mice has been carried out and it the first report from Assam and also from India.

Acknowledgments

The authors are grateful to the Dean, College of Veterinary Science, Assam Veterinary and Fishery University for providing necessary facilities to carry out the research work. They also declare that there is no conflict of interest related to the work.

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