



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2026): 4.82
 IJBS 2026; 8(3): 102-108
www.biologyjournal.net
 Received: 29-12-2025
 Accepted: 02-02-2026

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Prevalence of protozoan parasites inhabiting the digestive tract of domestic birds in Assam, India

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DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i3b.599>

Abstract

The present study was undertaken to ascertain the prevalence of protozoan parasites inhabiting the 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. The study was conducted in 8 districts of Assam viz. Kamrup (Rural and Metro), Dhubri, Barpeta, Nalbari, Darrang, Baksa, Lakhimpur and Dhemaji. A total of 1278 pooled faecal samples were collected for study.

Faecal examination by floatation method for the presence or absence of oocysts of coccidia and by Modified Ziehl-Neelsen staining for detection of *Cryptosporidium* infection was carried out in domestic birds and overall prevalence of protozoa was recorded as 34.66%. Identified species were *Eimeria labbeana* (26.35%), *E. columbarum* (8.69%) and *E. columbae* (3.53%) from pigeon; *Eimeria tenella* (19.13%), *E. acervulina* (9.46%), *E. necatrix* (6.88%), *E. maxima* (2.36%) and *Cryptosporidium baileyi* (3.01%) from chicken; *Eimeria battakhi* (19.86%) from duck and *Eimeria tsunodai* (13.29%), *E. bateri* (5.69%), *E. uzura* (3.16%) and *Cryptosporidium meleagridis* (4.43%) from quail.

Keywords: Pigeon, Chicken, Duck, Quail, Coccidia, *Cryptosporidium*

Introduction

Birds have been domesticated by humans from ancient times to provide supplementary income to most of the landless families. Among all the domestic birds, duck, chicken, geese, pigeons, quails, turkeys, etc., are more popular throughout the world. The world has over 23 billion poultry population, which counts at about three per person on the planet (FAOSTAT, 2016) ^[11] and about 5 times more than that of 50 years ago. They are kept and raised in a wide range of production systems to provide mainly meat and eggs and also manure for crop fertilization. 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. Quail meat has many nutrients such as protein, fat, calcium, iron and zinc and egg is a rich source of protein and choline that is very good for our brain.

Avian coccidiosis is a common protozoal disease known to be harmful to domestic birds in different countries including India (Dey *et al.*, 2010; Eljadar *et al.*, 2012) ^[9, 10]. It is caused by the genera *Eimeria*, *Isospora*, *Wenyonella* and *Tyzzeria*, with *Eimeria* spp. being more common in chicken and *Wenyonella* and *Tyzzeria* spp. reported to some extent in ducks. *Eimeria* spp. causes depression, loss of body weight, enteric disorders and mortality in the birds (Yang and Yang, 1995) ^[39]. Although the infection caused by the different species may produce haemorrhagic or non-haemorrhagic enteritis leading to a high level of mortality in a flock, the subclinical form is known to be economically more important due to impairment of gut health and feed utilization (Shirzad *et al.*, 2011) ^[32]. Moreover, the infected birds are predisposed to necrotic and other types of enteritis which have adverse impact on the flock performance and farm profitability (Shawkat *et al.*, 2015) ^[31]. One infected bird may expel few to hundreds of millions of oocysts per day which is the source of infection. Under suitable conditions of humidity, oxygen and temperature, they get sporulated, become invasive and able to infect birds. Cryptosporidiosis is another disease caused by the genus *Cryptosporidium* in humans, livestock, poultry, game birds and companion animals. The parasites infect epithelial cells in the microvillus border of the gastrointestinal tract of all

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vertebrates (O'Donoghue, 1995) [26] and additionally infect the bursa of Fabricius, respiratory system and other organs in birds (Nakamura and Meireles, 2015) [25].

Due to the perception of the insignificance of domestic birds, little attention in terms of research has been paid to these hosts in India. Limited and partial information on these protozoan parasites of domesticated birds in Assam being less documented, therefore the current study has been designed to provide information on these protozoan parasites.

Materials and Methods

The present study was undertaken to know the prevalence of protozoan parasites (species of coccidia and *Cryptosporidium*) inhabiting the digestive tract of domestic birds viz. pigeon (*Columba livia domestica*), chicken (*Gallus gallus domesticus*), duck (*Anas platyrhynchos domesticus*) and quail (*Coturnix coturnix japonica*) in 8 districts of Assam, India, viz. Kamrup (Rural and Metro), Dhubri, Barpeta, Nalbari, Darrang, Baksa, Lakhimpur and Dhemaji. Regular visits were made to different households, market places, commercial farms and temple premises to physically examine the birds for their health status, collection of samples (faeces of birds) for laboratory examination/ evaluation.

Faecal sample collection

A total of 1278 pooled faecal samples each of 2-5 g, from 368 pigeons, 465 chickens, 287 ducks and 158 quails were collected, examined microscopically by floatation method using Sheather's solution (sp. gravity 1.2) for the presence or absence of oocyst of coccidia (10X, 40X) and detection of refractile bodies of *Cryptosporidium* oocysts (40X, 100X). Faecal samples found positive for coccidia oocysts were mixed with 2.5% potassium dichromate solution for sporulation (Soulsby, 2012) [36].

Faecal smears prepared from individual samples were examined by the Modified Ziehl-Neelsen staining (Henriksen and Pohlenz, 1981) [15] for detection of *Cryptosporidium* oocysts which appear as pink to red stained spherical to ellipsoidal bodies containing four naked sporozoites against a pale green background under oil immersion objectives (100X). Species identification was done on the basis of host species, shape and size measured by micrometry (Sloss *et al.*, 1994) [35].

Analysis of data

The prevalence data obtained were subjected to statistical analysis using Chi-square test using SAS v.20 software.

Results and Discussion

Prevalence of coccidia infection

Out of 368 faecal samples from pigeon, 142 were found positive for coccidia (*Eimeria* sp.) with a prevalence of 38.58%. It was 37.84% in chicken, 24.04% in duck and 22.15% in quail. *Cryptosporidium* sp. was detected in 3.01% of chickens and 4.43% of quails, while none was recorded from pigeon and duck (Table 1).

(i) Prevalence of coccidia in pigeon

In pigeon, among the three species under genus *Eimeria*, oocysts of *Eimeria labbeana* were the most frequently detected (26.35%), followed by *E. columbarum* (8.69%) and

E. columbae (3.53%) as shown in Table 2 and Plate I. Statistical analysis by Chi square test revealed highly significant ($p < 0.01$) difference in prevalence of different species of *Eimeria*.

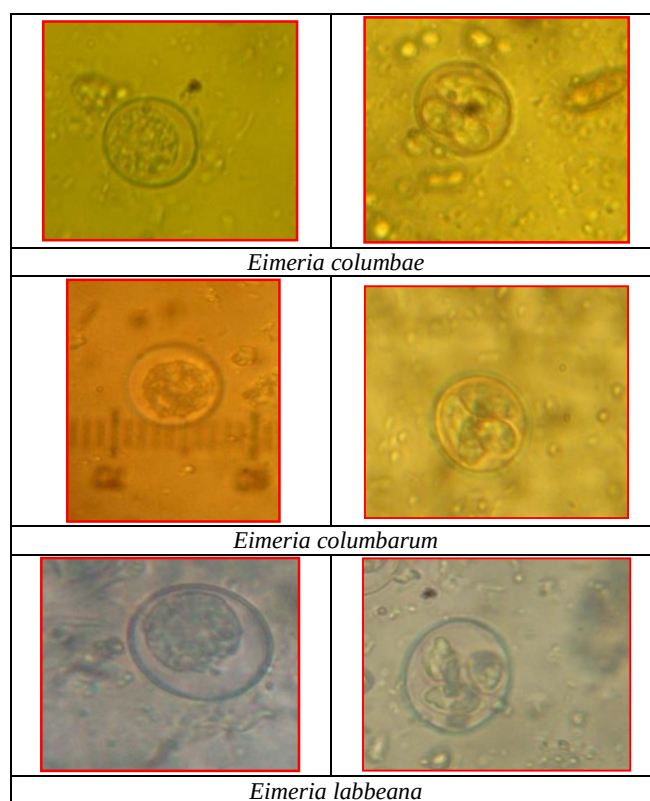


Plate 1: Unsporulated and sporulated oocyst of coccidia in pigeon, 400X

The morphological differences showing comparison among the three recorded *Eimeria* species from pigeon are presented in Table 3.

Overall detection of 38.58% coccidia infection caused by *Eimeria* sp. from pigeon during the present study tallies with reports of several workers from different countries such as 28.96% by Bandyopadhyay *et al.*, (2006) [6] and 31.0% by Sivajothi and Sudhakara Reddy (2015) [34] from India; 35% by Mahdii and Al-Rubaie (2013) [22] from Baghdad; 52% by Hui *et al.* (2012) [16] from Shanghai. Similarly, Saikia (2016) [29] in her study conducted in domestic pigeon from Assam reported 38.81% prevalence of coccidia infection. Aleksandra and Pilarczyk (2014) [3] similarly isolated three species (*Eimeria labbeana*, *E. columbarum* and *E. columbae*) in mixed infection in two pigeon lofts in West Pomerania. Saikia *et al.* (2017) [28] recorded four species viz. *Eimeria labbeana* (28.08%), *E. columbarum* (6.84%), *E. columbae* (2.86%) and *Eimeria* sp. (unidentified, resembling *E. duculai*) 1.14%, in domestic pigeon in Assam. Additionally, *Isospora* sp. (0.22%) was also reported. Highest detection of infection with *E. labbeana* similar to the present finding was reported by several workers (Hui *et al.*, 2012; Mahdii and Al-Rubaie 2013; Aleksandra and Pilarczyk, 2014; Yang *et al.*, 2016) [16, 22, 3, 40]. In the present study *Cryptosporidium* oocyst could not be detected in any of the faecal samples from pigeon. However, Faraj (2014) [12] recorded 40% prevalence of *Cryptosporidium* parasite from wild pigeons in Baghdad city and Bamaïyi *et al.* (2013) [5] recorded 7.4% in Nigeria.

Prevalence of coccidia and *Cryptosporidium* in chicken

In chicken, out of 465 faecal samples screened for coccidia, 190 were positive with overall prevalence of 40.86%. Statistical analysis revealed highly significant ($p < 0.01$) difference. Among four species of *Eimeria* (Plate II), oocysts of *Eimeria tenella* were most commonly detected (19.13%), followed by *E. acervulina* (9.46%), *E. necatrix*

(6.88%) and lowest prevalence of *E. maxima* (2.36%) as shown in Table 4. The *Cryptosporidium* oocyst isolated from chicken was identified as *C. baileyi*, which measured $4.6 \times 6.2 \mu\text{m}$ in size, with a prevalence of 3.01%. The morphological differences showing comparison among the four recorded *Eimeria* species from chicken are presented in Table 5.

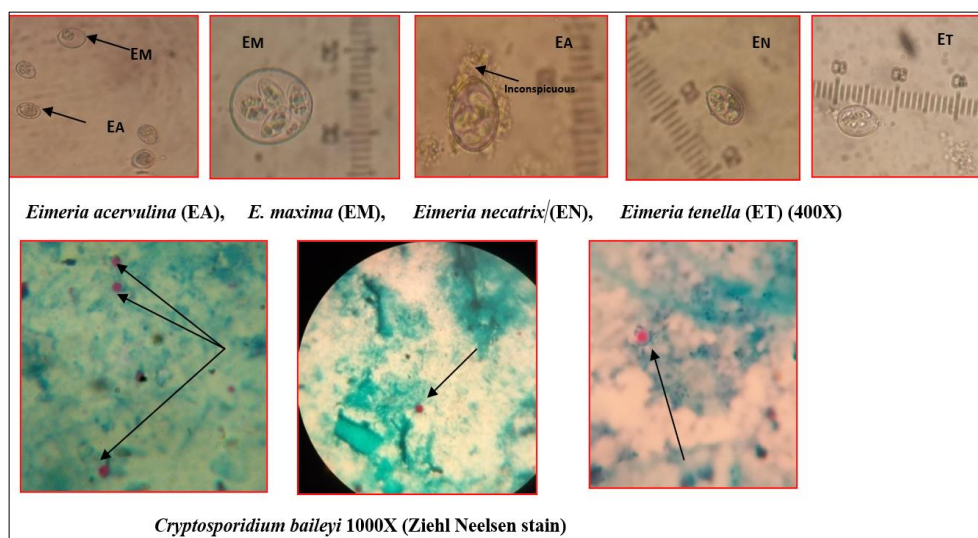


Plate 2: Coccidia and *Cryptosporidium* in chicken

The prevalence of 37.84% due to *Eimeria* spp. in chicken is almost similar to 31.8% observed by Gharekhani *et al.* (2014) [13] in Western Iran, 31.8% by Kaboudi *et al.* (2016) [19] in free-range chicken in Tunisia, 37.1% reported by Jatau *et al.* (2012) [18] in Zaria. Contrary to our findings, Luu *et al.* (2013) [21] found 56% prevalence of *Eimeria* in Ethiopia and Pant *et al.* (2018) [27] recorded 56.25% coccidiosis around Tarai region of Uttarakhand. The variation in prevalence might be attributed to different factors such as sampling periods, sample size, geographical area, and climatic conditions observed in the different study areas. Relatively high prevalence was reported in commercial farms compared to the backyard poultry farming which may be due to poor management, hygienic and sanitary practices such as perpetual spillage of water on the litters from the poultry drinkers, which encourages *Eimeria* build-up and subsequent sporulation and infection in the deep litter management. This is consistent with the reports by Methusela *et al.* (2002) [23] and Taylor *et al.* (2007) [37]. Kalita (2016) [20] recorded four *Eimeria* species in broiler chickens of Assam with highest prevalence of *E. tenella* (62.50%) followed by *E. acervulina* (23.43%), *E. mitis* (12.50%) and *E. maxima* (1.56%). *E. tenella* as the most common species was recorded by many workers from India

(77% by Singh *et al.*, 1977 [33] from Assam; 43.59% by Jadhav *et al.*, 2012 [17] from Maharashtra) and abroad (Hadipour *et al.*, 2011 [14] from Southwest Iran, Kaboudi *et al.*, 2016 [19] from Tunisia).

Current *et al.* (1986) [8] reported *Cryptosporidium baileyi* as the most frequently diagnosed species with reports of clinical or subclinical disease in 12 avian orders including Galliformes. In our present study, the chicken positive for *Cryptosporidium* oocysts appeared healthy without any signs of illness. This agreed with the report of Blagburn *et al.* (1987) [7] who observed that oral infection by *C. baileyi* is generally subclinical, although there may be decreased weight gain, which may only be transitory and drinking water might serve as a main source of distribution of oocysts among poultry flocks.

Prevalence of coccidia in duck

Two species of *Eimeria* viz. *Eimeria battakhi* (19.86%) and unidentified *Eimeria* sp. (4.18%), were recorded in ducks out of 287 pooled faecal samples examined with an overall prevalence of 24.04% (Table 6, Plate III). Statistically, the difference was highly significant ($p < 0.01$). No *Cryptosporidium* sp. was found. The morphological difference of the two *Eimeria* species is shown in Table 7.

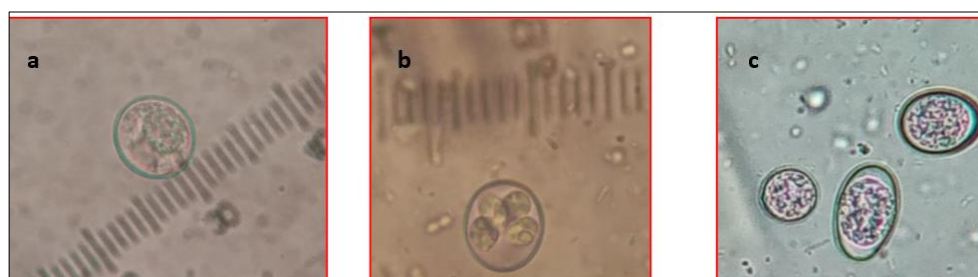


Plate 3: Unsporulated and sporulated oocyst of *Eimeria battakhi* (a-b); unidentified *Eimeria* sp. (c) in duck, 400X

Scanty literature about *Eimeria* spp. in ducks is available to compare with our present result. Nagwa *et al.* (2013) ^[24] in their study in Gharbia Governorate, Egypt, recorded 4.5% *Eimeria* and identified three species- *E. battakhi* (1.7%), *E. danailova* (1%) and *E. schachdagica* (1.2%) in single or mixed infection. Zuo *et al.* (1990) ^[41] also recorded *E. battakhi* in China similar to our findings. Adejinmi *et al.* (2011) ^[1] recorded a high prevalence of *Eimeria* sp. (34.3%) along with *Tyzzeria* sp. (16.6%) and *Cryptosporidium* sp. (15.4%) by examining 175 faecal samples of domestic ducks (*Anas platyrhynchos*) in Nigeria.

Prevalence of coccidia and *Cryptosporidium* in quail

A total of 158 pooled faecal samples from quail were

screened for coccidia and 42 were found positive with an overall prevalence of 26.58%. Three species of *Eimeria* i.e. *Eimeria bateri* (5.69%), *E. uzura* (3.16%) and *E. tsunodai* (13.29%) either as single or in mixed infection, were recorded. One species of *Cryptosporidium*, i.e. *C. meleagridis* (4.5-4.0 × 4.6-5.2 µm) was reported with a prevalence of 4.43% (Table 8 and Plate IV). Statistically, highly significant ($p < 0.01$) difference was seen. The morphological differences showing comparison among the three recorded *Eimeria* species from quail are presented in Table 9.

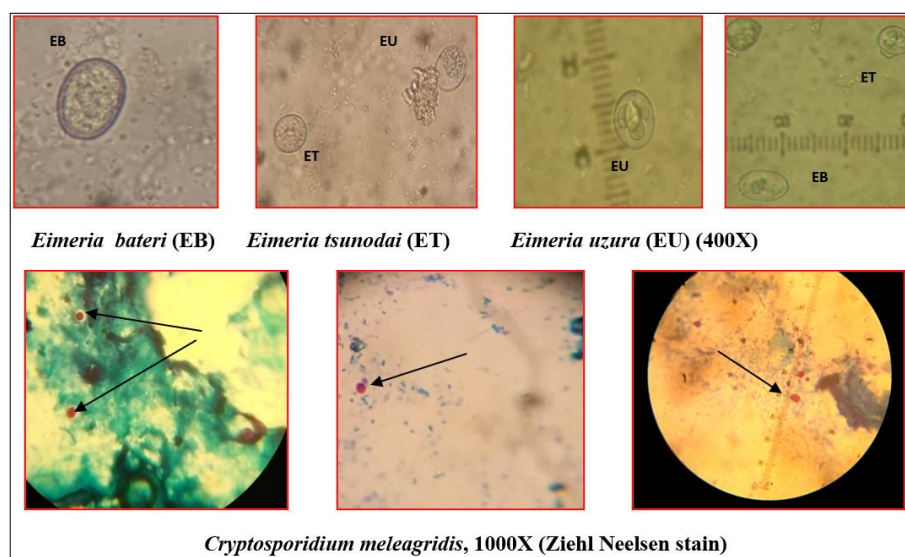


Plate 4: Coccidia (*Eimeria*) and *Cryptosporidium* oocyst in quail

Similar to our findings, Anbarasi *et al.* (2016) ^[4] recorded 15.78% prevalence of *Eimeria* spp. due to *E. tsunodai*, *E. bateri* and *E. uzura* in Japanese quail in Namakkal and Coimbatore districts from Chennai, India. However, a higher prevalence rate was reported by Al Saeedy and Al Rubaie (2014) ^[2] in Baghdad (78.33%). Teixeira *et al.* (2004) ^[38] identified *E. tsunodai*, *E. bateri* and *E. uzura* from young and mature Japanese quails of either sex in Rio de Janeiro, Brazil. Most of the authors reported highest

prevalence of *Eimeria tsunodai* compared to the other *Eimeria* species as was observed in our present study. Shaapan *et al.* (2011) ^[30] recorded small-sized (5.2 × 4.4 µm) round or slightly ovoid oocysts of *C. meleagridis* from native quails in different farms in Giza, Egypt similar to our findings. Lethargy, decreased body weight gain, lower pigmentation, and diarrhea were the most common clinical signs.

Table 1: Overall prevalence of G.I protozoa in domestic birds by faecal examination

Host	No. of faecal sample examined	Species of protozoan parasite		Total
		<i>Eimeria</i> sp. (%)	<i>Cryptosporidium</i> sp. (%)	
Pigeon	368	142 (38.58%)	0 (0.0%)	142 (38.58%)
Chicken	465	176 (37.84%)	14 (3.01%)	190 (40.86%)
Duck	287	69 (24.04%)	0 (0.0%)	69 (24.04%)
Quail	158	35 (22.15%)	7 (4.43%)	42 (26.58%)
Total	1278	422 (33.02%)	21 (1.64%)	443 (34.66%)
Chi square value		2487.56	0.729 ^{NS}	
P value		< 0.001	0.393	

Highly significant $P < 0.01$, ^{NS} Non -significant $p > 0.05$.

Table 2: Species wise prevalence of coccidia in pigeon

Species of coccidia identified	Sample examined	No of positive sample	Prevalence (%)	Chi square value	P value
<i>Eimeria labbeana</i>	368	97	26.35	94.09**	< 0.001
<i>E. columbarum</i>		32	8.69		
<i>E. columbae</i>		13	3.53		
Total	368	142	38.58		

Highly significant, ($p < 0.01$)

Table 3: Comparative morphology of *Eimeria* spp. oocyst detected in pigeon

Characters	<i>E. labbeana</i>	<i>E. columbarum</i>	<i>E. columbae</i>
Oocyst shape	slightly spherical to subspherical	spherical or subspherical	Subspherical
Size (µm)	19.5-23.43×16.41-19.03 µm	20.06 × 17.52 µm	16.4 × 14.3 µm
Micropyle	No visible micropyle	Absent	Absent
Sporocyst shape	elongate ovoid	elliptical or elongated	Ellipsoidal
Sporulation time (hour)	24-36 hours	36-40 hours	65-72 hours

Table 4: Species wise prevalence of coccidia and *Cryptosporidium* in chicken

Species of coccidia identified	Sample examined	No of samples positive	Prevalence (%)	Chi square value	p value
<i>Eimeria acervulina</i>	465	44	9.46	114.00**	< 0.001
<i>Eimeria necatrix</i>		32	6.88		
<i>Eimeria tenella</i>		89	19.13		
<i>Eimeria maxima</i>		11	2.36		
<i>Cryptosporidium baileyi</i>		14	3.01		
Total	465	190	40.86		

Highly significant ($p < 0.01$)**Table 5:** Comparative morphology of *Eimeria* spp. oocyst detected in the chicken

Characters	<i>E. acervulina</i>	<i>E. necatrix</i>	<i>E. tenella</i>	<i>E. maxima</i>
Oocyst shape and Index-Length width	Egg-shaped or small ovoid 1.25	Broadly ovoid 1.19	Broadly ovoid in shape 1.16	ovoid, egg-shaped or ellipsoidal 1.47
Size (µm)	12-23× 9-17 µm	15.5 -23.3 µm × 13.6 - 20 µm	19.2 -26 µm × 16 -22 µm	21 µm -42 µm × 16 µm -30 µm
Micropyle	Inconspicuous micropyle	Absent	Absent	Absent
Oocyst residuum	Absent	Absent	Absent	Absent
Sporocyst residuum	Present	Absent	Absent	Absent
Sporulation time (hour)	18 hours	48 hours	24-48 hours	30 hours

Table 6: Species wise prevalence of coccidia in duck

Species of coccidia identified	Sample examined	No of positive sample	Prevalence (%)	Chi square value	p value
<i>Eimeria battakhi</i>	287	57	19.86	33.36**	<0.001
Unidentified <i>Eimeria</i> sp.		12	4.18		
Total	287	69	24.04		

Highly significant ($p < 0.01$)**Table 7:** Comparative morphology of coccidia oocyst detected in the duck

Characters	<i>E. battakhi</i>	Unidentified <i>Eimeria</i> sp.
Oocyst shape	Subspherical to ovoid	Oval and spherical
Oocyst wall	Smooth	Smooth
Size (µm)	19.5 × 21 µm	17 × 10 µm
Micropyle	Absent	Absent
Oocyst residuum	Absent	Absent
Sporocyst residuum	Present	Absent
Sporulation time	24-48 hours	-

Table 8: Species wise prevalence of coccidia and *Cryptosporidium* in quail

Species of coccidia identified	No. of Samples examined	No of positive sample	Prevalence%	Chi square value	p value
<i>Eimeria bateri</i>	158	9	5.69	15.81**	0.001
<i>Eimeria uzura</i>		5	3.16		
<i>Eimeria tsunodai</i>		21	13.29		
<i>Cryptosporidium meleagridis</i>		7	4.43		
Total	158	42	26.58		

Highly significant $p < 0.01$ **Table 9: Comparative morphology of *Eimeria* spp. oocyst detected in Japanese quails

Characters	<i>E. bateri</i>	<i>E. uzura</i>	<i>E. tsunodai</i>
Oocyst shape	Subspherical, ovoid or ellipsoid	Ovoid	Ovoid
Size (µm)	22.30 ± 1.84 × 16.76 ± 1.32 µm	21.48 ± 6.76 × 18.23 ± 1.13 µm	17.23 ± 1.5 × 14.88 ± 0.79 µm
Micropyle	Absent	Absent	Absent
Oocyst residuum	Absent	Absent	Absent
Sporocyst residuum	Present	Present	Present
Sporulation time	24-48 hours	36-48 hours	22-36 hours

Conclusion

From the present study it could be concluded that digestive tract protozoan infection in domestic birds is predominant in Assam. Four species of coccidia, viz. *E. tsunodai*, *E. bateri*, and *E. uzura* from quail; *E. battakhi* from duck and two species of *Cryptosporidium* i.e. *Cryptosporidium baileyi* from chicken and *Cryptosporidium meleagridis* from quail represent the first report from Assam.

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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