

Diversity of *Eimeria* Schneider, 1875 (Conoidasida: Eucoccidiorida: Eimeriidae) in Broiler chickens of Purulia District, West Bengal, India

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Abstract

Eimeria is one of the most important pathogenic parasites in broiler chickens that causes huge economic loss in poultry birds throughout the world including the Purulia district of West Bengal. To study the prevalence of coccidiosis in the broiler chickens of the district, coprological studies were carried out from February 2022 to January 2023. Faecal samples were collected from different poultry farms of the district and examined by the Floatation method (Sheather's solution) followed by microscopic examination of coccidian oocyst and sporocyst. Species were identified by morphometric analysis and standard literature. Out of 254 samples examined, 169 samples were found positive for coccidian species, i.e. the prevalence of coccidiosis in broiler chickens of the Purulia district was 66.53%. Altogether six species of *Eimeria* were recorded of which *Eimeria tenella* was the most prevalent species (40.55%, 103/254), followed by *Eimeria necatrix* (12.20%, 31/254), *Eimeria maxima* (5.51%, 14/254), *Eimeria mitis* (3.54%, 9/254), *Eimeria acervulina* (2.75%, 7/254) and *Eimeria brunetti* (1.96%, 5/254). The most common type of mixed infection was found in combination with *E. tenella*, *E. maxima* and *E. necatrix* (33.85%, 86/254), followed by *E. tenella*, *E. maxima* (25.19%, 64/254). Interestingly, in most cases, *E. tenella*, *E. maxima* and *E. necatrix* were coexisting in various combinations. *E. tenella*, *E. maxima* and *E. necatrix* were coexist in 33.85%, (86/254) cases, *E. tenella* and *E. necatrix* in 25.19% cases (64/254) and *E. tenella* and *E. maxima* in 15.35% cases (39/254). The present study also revealed that the infection was more prevalent in the rainy season (91.78%) and less in the summer (31.81%). The present communication intends to conclude that coccidiosis is still a major threat to poor poultry bird keepers of the district.

Keywords: Oocyst, *Eimeria*, Sporocyst, Poultry, Prevalence

Introduction

Chickens represent the biggest poultry sector in the world that reared extensively and represent a good, cheap, and healthy protein source for the common people. India ranked 3rd in egg production and 8th in meat production in the world (2021-22 report of The Economic Times news, 31 January 2022). Poultry bird keeping is one of the fewest options for livelihood development of the rural people of any developing country like India. The poultry sector generates more than 3 million employment and it is expected to cross 5 million

by 2025 in India (As per data from Central Avian Research Institute). However, rural poultry birds keepers often succumb to huge economic losses due to sudden outbreaks of diseases. Coccidiosis is one such disease which causes loss of millions of dollars every year throughout the globe (Dalloul RA et., al 2006). A study in 2017, indicates 76% and 79.4% of farms from southern and northern India were found to be infected for any species of *Eimeria* (Prakash babu BC et al., 2017). It is a kind of severe gastrointestinal disease of poultry birds caused by several species of protozoan

obligate endoparasitic genera *Eimeria* under the phylum Apicomplexa. Globally seven pathogenic *Eimeria* species infective to chickens are identified viz. *E. tenella*, *E. necatrix*, *E. maxima*, *E. mitis*, *E. acervulina*, *E. brunetti* and *E. praecox* (Carolina Mesa-Pineda *et al.*, 2021). The most common and highly pathogenic *Eimeria* species in the poultry industry is the *E. tenella* (Ayaz *et al.*, 2003) whereas, *E. praecox* is the least pathogenic (Carolina Mesa-Pineda *et al.*, 2021). Coccidiosis is endemic in most the tropical regions because of favorable ecological conditions for sporulation of coccidian oocyst and its subsequent development and poor management conditions (Blake and Tomley, 2014). The prevalence of coccidiosis in broiler chickens mostly depend upon the age of the chicken (Sharma *et al.*, 2015). Young birds are more prevalent and more readily display to show the symptoms, whereas matured or adult chickens are relatively more resistant (Cervantes *et al.*, 2020). This disease is more severe in broiler chickens where the rate of mortality and morbidity is very high. Dysentery, diarrhoea, and enteritis, which can result in bloody stools in some *Eimeria* species, emaciation, feed conversion that is below average, delayed sexual maturity, drooping wings that cause dehydration, poor development, and low egg and meat quality production are all symptoms of coccidiosis. (Rehman *et al.*, 2010, Abbas *et al.*, 2017). There is hardly any serious work on the Coccidiosis of poultry birds in Purulia district, West Bengal, where thousands of poor rural people are depending on rearing of chickens as primary or secondary source of their income. The aims of the present study is to find out the prevalence and diversity of Coccidiosis of poultry chickens in the Purulia district.

Materials and Methods

Study area

This present study was conducted in every block of the Purulia district of West Bengal, The geographical location of Purulia district range between 22° and 23.50° N latitudes and 85.75° and 86.65°E east longitudes. The geographical area of the Purulia district is around 6,259 -kilometer square (Figure 1).

Study period

The present research work was conducted from February 2022 to January 2023 to explore the prevalence of Coccidiosis in poultry chicken of the Purulia district.

Collection of samples

To determine the prevalence of coccidia infection in broiler chickens, a total of 254 pooled faecal samples of poultry were collected from various poultry farms in the current study region. About 3-5 g of each sample were collected in distinct screw-capped vials with proper labeling and brought to the laboratory. The collected faeces samples were evaluated either that day or after being stored in a refrigerator at 4°C for further investigation. The samples of faeces were first thoroughly examined to determine their consistency and the presence of mucous, blood, etc. Standard techniques were used to determine the presence of Eimerian oocysts.

According to the approach described by Soulsby (1982), sucrose solution (also known as Sheather's solution) was used to isolate the coccidia oocysts.

Sporulation of coccidia oocyst

Faecal samples which were positive for coccidia oocysts after the sugar floatation method were mixed with 2.5% potassium dichromate solution in Petri dishes and left at room temperature for sporulation of oocysts. Coccidia species were identified according to their size and morphological features of the oocysts (Saikia *et al.*, 2017). The thickness of the oocyst walls, the presence of micropyle, cap, polar granules, size and shape of the sporocysts, the thickness of sporocyst wall, the shape of the Stieda bodies. and sporulation time (Soulsby, 1982) were recorded. Measurements of 25 oocysts of each samples are measured. Measurement of different coccidian oocysts and sporocysts were examined as per the procedure described by Sloss *et al.* (1994).

Systematic accounts of *Eimeria*, Schneider, 1875

Phylum – Apicomplexa Levine, 1970

Class -Conoidasida Levine, 1988

Order-Eucoccidiorida Leger and Duboscq, 1910

Family-Eimeriidae Minchin, 1903

Genus – *Eimeria* Schneider, 1875

Results and Discussion

In this present study out of 254 faecal samples, 169 samples were found to be infected with *Eimeria* species and the overall prevalence rate of infection was 66.53% (Table 1)). Season wise data shows that the highest prevalence of Coccidiosis

in Poultry chickens in the Purulia district is 91.78% in monsoon and lowest in summer with 31.81% (Figure 3). Month wise data shows that the highest rate of infection occurred in the month of July (96.6%) and the lowest in the month of April (20.0%) (Table 3). The monthly prevalence rate is overall high during the month of July-January, after that it falls sharply and continues up to June.

Considering the measurement of the oocyst and variation of sporulation time, six species of *Eimeria* were identified in broiler chicken of Purulia. Among the six species of the genus *Eimeria*, oocyst of *Eimeria tenella* was most prevalent (40.55%, 103/254) and it is followed by *E. necatrix* (12.20%, 31/254), *E. maxima* (5.51%, 14/254), *E. mitis* (3.54%, 9/254), *E. acervulina* (2.75%, 7/254), and *E. brunetti* (1.96%, 5/254).

Description of oocyst

Eimeria tenella Tyzzer, 1929, (Figure 2A)

The oocysts are broad and oval in shape. The Oocyst wall is two layered, 1.2 µm thick. The outer layer is thick, the inner layer is thinner. Micropyle and micropylar cap are absent. The unsporulated oocyst shows an oval to subspherical sporoblast filling the central portion of the oocyst. Oocystic residuum is absent. The oocyst size ranges from 18.7-22.2 µm in length and 15.3-18.8 µm in width. The sporocysts are broad and elongated and slightly tapering at the anterior portion and the posterior end of the sporocyst is rounded, and broad. The sporocyst ranges from 10.19-11.0 µm in length and 5.9-7.0 µm in width. Anterior end is tapering with a stieda body. Sporocystic residuum is absent. The sporulation time is 24-72 hours, and the prevalence rate of *E. tenella* is 40.55%.

Eimeria necatrix Johnson, 1930, (Figure 2B)

The oocysts are round and oval in shape and covered by a double-layered wall. The outer wall is thick and light yellow in color while the inner wall is thin and brownish to whitish. Thickness of the oocyst wall is about 1.3µm. Micropyle and micropylar cap are absent. The un-sporulated oocyst shows a small spherical sporoblast filling the central portion of the oocyst. The sporulated oocyst shows the presence of clear polar granules at the anterior end and behind the oocyst wall. No oocystic residuum is seen. The oocyst size ranges from 13.1-22.2 µm in length and 11-18.6 µm in width. The sporocysts are mainly comma in shape, measuring about 8.21-13.58 µm in length and 4.90-6.20 µm in width. The posterior portion of the sporocyst is rounded, and broader,

anterior end is narrow, tapering with large size stieda body. The sporulation time varied between 16-48 hours. The rate of prevalence of *E. necatrix* is 12.20%..

Eimeria maxima Tyzzer, 1929, (Figure 2C)

The oocysts of the species collected from the broiler chicken are oval to egg-shaped without micropyle and micropylar cap. The oocyst wall is two layered, and about 1.2 µm thick. The outer wall is thick, and reddish brown while the inner is thin and bluish in colour. The sporulated oocyst has polar granules along the oocyst wall at the anterior end. Oocystic residuum is absent. The sporocysts are elongated, the anterior end is pointed with a clear steida body and the posterior end is broad and rounded. The oocyst measures 21.4-42.8 µm in length and 16.43-29.0 µm in width. Each sporocyst measures about 8.16-12.24 µm in length and 5.0 - 5.2 µm in width. Sporulation time varied from 24-36 hours. The prevalence rate of *E. maxima* is 5.51% in the present study.

Eimeria mitis Tyzzer, 1929, (Figure 2D)

The identified oocysts are spherical to oval in shape and covered with a thin dark brownish-coloured oocyst wall. *E. mitis* has a single oocyst wall, measured about 0.9 µm thick. Micropyle and micropylar cap is absent. The un-sporulated oocyst shows a spherical granular sporoblast with entire portion of the oocyst. The sporulated oocyst shows the clear polar granule near the oocyst wall. Oocystic residuum is absent. The sporocysts are elongated, oval to egg shaped, and show typical arrangement within the oocyst. The oocyst measures 14.4-19.48 µm in length and 13.34-16.90 µm in width. Sporocyst measures about 8.21-12.26 µm in length and 4.7-5.9 µm in width. The posterior end of the sporocyst is rounded, and large, anterior end is narrow and clear with the stieda body. Sporulation time varied from 16- 24 hours. The prevalence rate of *E. mitis* is 3.54%.

Eimeria acervulina Tyzzer, 1929, (Figure 2E)

The oocysts of this species are ellipsoidal in shape. It has no micropyle and micropylar cap in the oocyst. The oocyst wall is about 1.2 µm thick. It is two layered, the outer wall is thick, and light yellow in colour but the inner wall is thin and brown in colour. The un-sporulated oocyst shows an oval spherical sporoblast at the center of the oocyst. A distinct polar granule is visible at the anterior end of the sporulated oocyst. The oocyst length varies from 18.12-26.9 µm in length and 13.2-20.24 µm in width. The sporocysts are typically elongated to pyriform in shape, measuring 10.18-13.24 µm in length and 5.1-6.4 µm in width. The posterior

portion of the sporocyst is broader in shape, anterior end is narrow with clear stieda body. The sporulation time is 24-48 hours and the prevalence rate of infections is 2.75%.

***Eimeria brunetti* Levine, 1942, (Figure 2F)**

The oocysts are spherical to oval in shape. It has no micropyle and micropylar cap. The wall of the oocyst is single-layered, 1.3 µm thick, and brown in colour. The un-sporulated oocyst shows a spherical sporoblast presenting a small portion of the oocyst. The sporulated oocyst shows the presence of a clear polar granule at the anterior end near to the oocyst wall. Oocystic residuum is not present. The oocyst length ranges from 19.2-23.4 µm and 13.5-19.4 µm in width. The sporocysts are elongated in shape, and measure about 8.14-13.34 µm in length and 5.0-6.3 µm in width. The posterior end of the sporocyst is round and broader, the anterior end is narrow with clear stieda body. Sporocystic residuum is absent. The sporulation time of *E. brunetti* is varies from 18-24 hours and the prevalence rate of infections is 1.96%.

Comparative characteristics of six *Eimeria* species, found in poultry chicken of Purulia district, are compiled in Table 2.

The overall prevalence of infection of Coccidiosis in Poultry chickens of the Purulia district is 66.63% and similar kind of observations are recorded by Gari *et al.*, (2008), Olanrewaju and Agbor (2014) in Nigeria. Dinka and Tollosa (2012) in Ethiopia. However Oljira *et al.*, (2012) reported a much lower rate of prevalence (20.57%) from Ambo in Ethiopia.

The present study confirms the persistent occurrence of coccidiosis with seasonal variation in prevalence. Though the incidence rate was recorded as high during the monsoon period but highest in the month of July because of the favourable environmental conditions like high relative humidity (75%), 20°C-30°C degree centigrade temperature, and rainfall of about 600mm. The lowest infection was recorded during summer with the lowest in April, mostly because of extreme weather conditions like very low

relative humidity, temperature and rainfall which measured 20-35%, 35°C-40°C and 50 mm respectively. Statistical analysis by two-way ANOVA results (P-value 0.00076) revealed that there is a significant ($p < 0.05$) difference in the prevalence of different *Eimeria* species and their infection in different seasons (particularly monsoon to summer and spring) clearly shows that prevalence of infection high in monsoon compared to summer and spring (Table 5). The present investigation finds a strong correlation between the occurrence of coccidiosis and environmental conditions. Similar kinds of observations are also noticed by Haug *et al.*, (2008), Sharma *et al.*, (2013). They also pointed out that high humidity and rainfall favoured the rate of sporulation of coccidian oocysts.

Conclusion

This present study revealed that *E. tenella* was the most prevalent species (40.55%) followed by *E. necatrix* 12.20% . *E. acervulina* and *E. burnetti* were the least prevalent species with a prevalence rate of 2.75% and 1.96% respectively (Table 4). The present study indicates that the highest rate of coccidiosis infection mostly occurred in monsoon (91.78%) and winter (88.15%) and the least infection noticed in spring (35.9%) and summer (31.81%) (Figure 3). Though coccidiosis in poultry is prevalent throughout the year, it varies with the change in climatic conditions like temperature, rainfall, humidity, and habitat stress (poor management) of the Poultry. The findings of the present study stressed on the regular surveillance of poultry birds for coccidiosis so that the poor poultry bird keeper of the district can be saved from huge economic loss. Disinfection assumes a significant part in diminishing the dispersal of the parasite (Peek HW, 2010), as the most continuous method of transmission of oocysts is through carriers like the movement of staff or instruments among the farms, and the presence of rodents and other insect pests (Cervantes HM *et al.*, 2020).

Table 1. Prevalence of coccidiosis in Poultry chickens of the Purulia district

Sample examined	Species of coccidia (<i>Eimeria</i>) identified	No. of positive samples	Prevalence (%)
254	<i>Eimeria tenella</i> Tyzzer, 1929	103	40.55
	<i>E. necatrix</i> Johnson, 1930	31	12.20
	<i>E. maxima</i> Tyzzer, 1929	14	5.51
	<i>E. mitis</i> Tyzzer, 1929	9	3.54
	<i>E. acervulina</i> Tyzzer, 1929	7	2.75
	<i>E. brunetti</i> Levine, 1942	5	1.96
Total 254		169	66.53

Table 2. Comparative features of six *Eimeria* species in poultry chicken of the Purulia district

Species/features	<i>Eimeria tenella</i> Tyzzer, 1929	<i>E. necatrix</i> Johnson, 1930	<i>E. maxima</i> Tyzzer, 1929	<i>E. mitis</i> Tyzzer, 1929	<i>E. acervulina</i> , Tyzzer, 1929	<i>E. brunetti</i> Levine, 1942
Oocyst shape	oval	oval	egg shaped	spherical	ellipsoid	spherical
Measurement in μm	18.7-22.2×15.3-18.8	13.1-22.4×11-18.6	21.4-42.4×16.43-29	14.4-19.4×13.34-1690	18.12-26.9×13.2-20.44	19.2-23.4×13.5-19.4
Micropyle	absent	absent	absent	absent	absent	absent
Micropylar cap	absent	absent	absent	absent	absent	absent
Polar granules	present	present	present	present	present	present
Sporocyst shape	elongated	pyriform	ovoid	egg shaped	like small banana	elongated
Stieda body	present	present	present	present	present	present
Measurement of sporocyst in μm	10.19-11×5.9-7.0	8.21-13.58×4.90-6.20	8.16-12.24×5-5.2	8.21-12.26×4.71-5.9	10.18-13.4×5.1-6.4	8.14-13.34×5.0-6.3
Sporulation time (hours)	24-72	16-48	24=36	16-24	24-48	18-24
Oocyst residuum	absent	absent	absent	absent	absent	absent

Table 3. Month-wise prevalence of *Eimeria* in chickens of the Purulia district.

Sl No.	Study period	Number of samples examined	No of samples positive	Prevalence (%)
1	February	21	9	42.85
2	March	18	5	27.8
3	April	20	4	20.0
4	May	19	7	36.8
5	June	27	10	37.03
6	July	29	28	96.6
7	August	23	21	91.30
8	September	21	18	85.7
9	October	20	18	90
10	November	20	17	85
11	December	16	14	87.5
12	January	20	18	90
	Total	254	169	66.53%

Table 4. Species (parasite) wise prevalence of infection in Poultry chicken of the Purulia district

Species name	Summer (Infected/examined ×100)	Monsoon (Infected/examined ×100)	Winter (Infected/examined ×100)	Spring ((Infected/examined ×100)	Total prevalence (%)
<i>Eimeria tenella</i>	11/66 (16.7%)	40/73 (54.8%)	46/76 (60.53%)	6/39 (15.4%)	103/254 (40.55%)
<i>E. necatrix</i>	6/66 (9.09%)	12/73 (16.43%)	10/76 (13.16%)	3/39 (7.7%)	31/254 (12.20%)
<i>E. maxima</i>	2/66 (3.03%)	5/73 (6.84%)	6/76 (7.9%)	1/39 (2.6%)	14/254 (5.51%)
<i>E. mitis</i>	0	2/73 (2.73%)	4/76 (5.3%)	3/39 (7.7%)	9/254 (3.54%)
<i>E. acervulina</i>	1/66 (1.51%)	5/73 (6.84%)	0	1/39 (2.6%)	7/254 (2.75%)
<i>E. brunetti</i>	1/66 (1.51%)	3/73 (4.11%)	1/76 (1.31%)	0	5/254 (1.96%)
Total	31.81%	91.78%	88.15%	35.9%	66.53%

Table 5. Showing the Anova result

SUMMARY	Count	Sum	Average	Variance
<i>Eimeria tenella</i>	4	147.43	36.8575	583.0232
<i>E. necatrix</i>	4	46.38	11.595	15.7575
<i>E. maxima</i>	4	20.37	5.0925	7.134092
<i>E. mitis</i>	4	15.73	3.9325	10.99156
<i>E. acervulina</i>	4	10.95	2.7375	8.616692
<i>E. brunetti</i>	4	6.93	1.7325	2.960692
Summer	6	31.84	5.306667	41.25899
monsoon	6	91.75	15.29167	397.5781
Winter	6	88.2	14.7	526.5485
Spring	6	36	6	30.652

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	3620.923	5	724.1846	7.991649	0.000759	2.901295
Columns	526.1862	3	175.3954	1.935554	0.167233	3.287382
Error	1359.265	15	90.61767			
Total	5506.374	23				

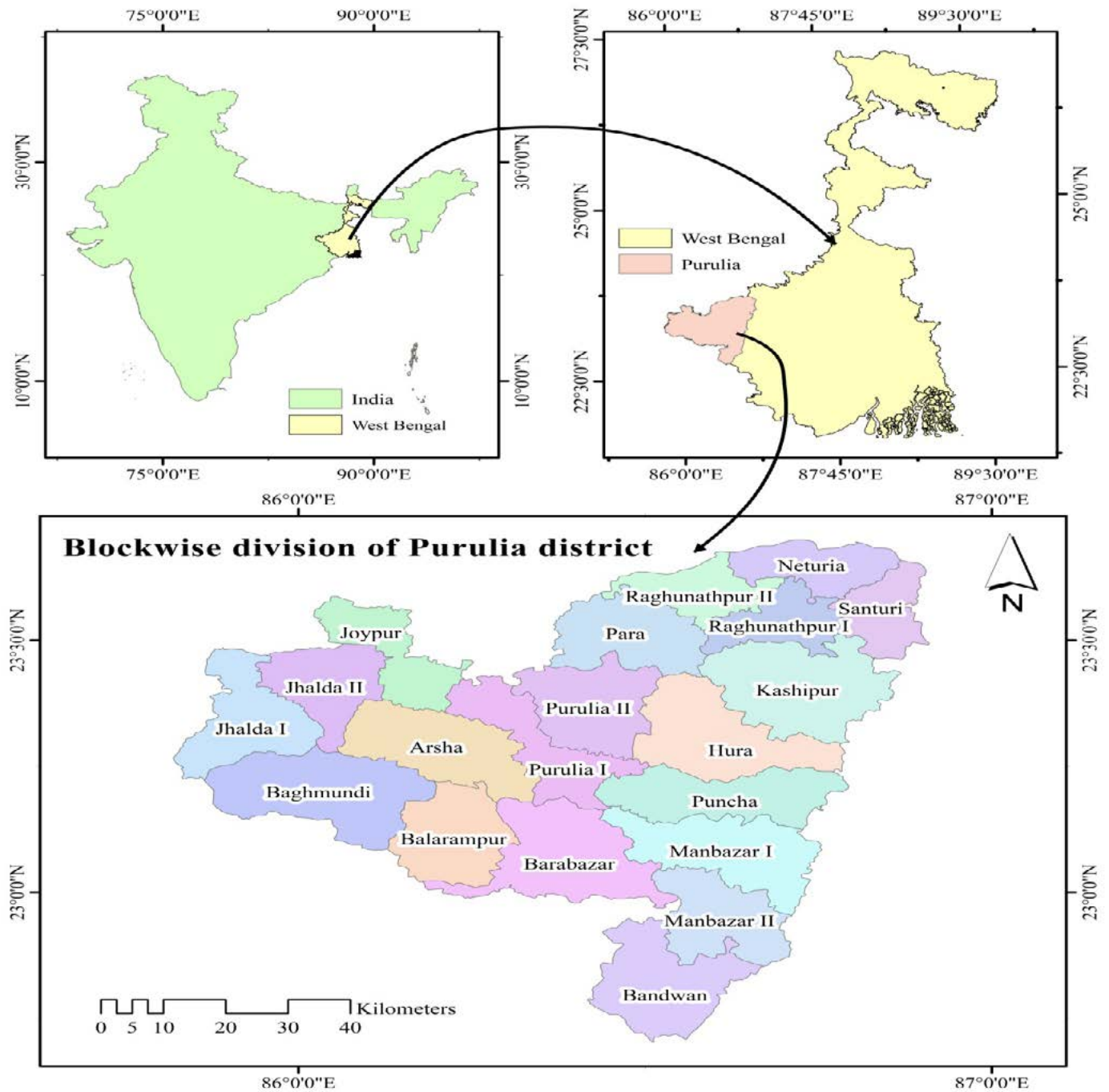


Figure 1 Map showing the study area.

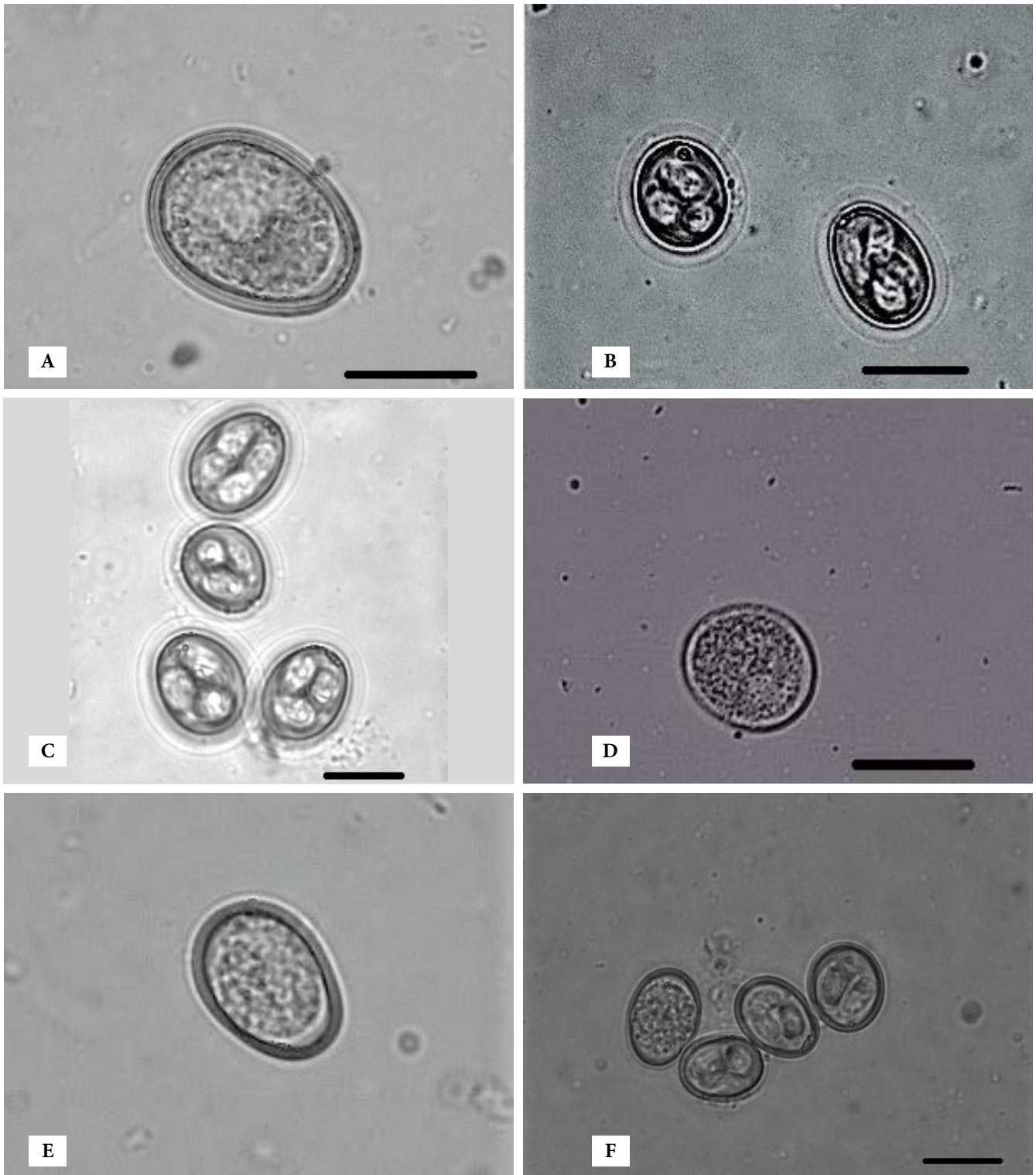


Figure 2: Species of *Eimeria* found in the Purulia district A. Oocyst of *Eimeria tenella* B. Oocyst of *Eimeria necatrix* C. Oocyst of *Eimeria maxima* D. Oocyst of *Eimeria mitis* E. Oocyst of *Eimeria acervulina* F. Oocyst of *Eimeria brunetti*.

(Bar = 10 μ m)

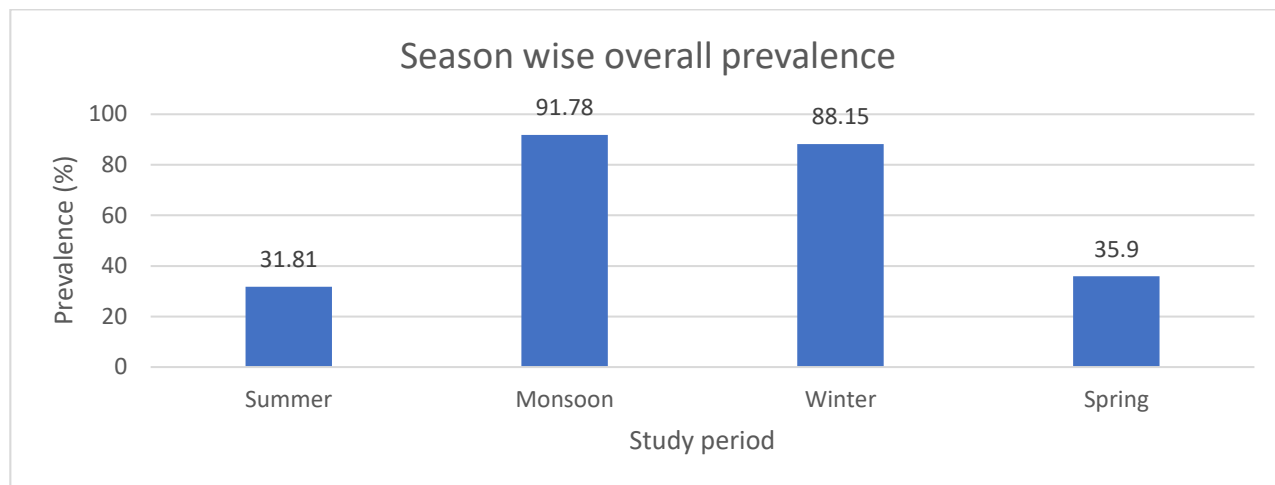


Figure 3 Seasonal prevalence of coccidiosis in poultry chicken of the Purulia district

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