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Prevalence and intensity of infection of different Eimeria species in Broiler chicken, *Gallus gallus* domesticus from Imphal, Manipur, India

KEYWORDS: Broiler chicken, Eimeria species, Intensity of infection, Prevalence, Manipur

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ABSTRACT *Intestinal parasitic infections in birds are constantly countered, even when they occur in low amounts, may result in subclinical diseases. The report is based after investigating the prevalence of intestinal parasitic infections in birds in Manipur. A cross-sectional study was conducted between June 2011 and June 2012. 150 fresh faecal samples of broilers were collected from 7 commercial farms. The samples screened for intestinal parasitic infection on the Eimeria species of Broiler chicken, Gallus gallus domesticus in Imphal, Manipur revealed the presence of five species – Eimeria tenella, E. maxima, E. mitis, E. acervulina and E. praecox. Morphological descriptions and photomicrographs are provided. Infection intensity was higher in mud floored farms than in cement floored farms. Younger birds are more susceptible to infection. Among the species of Eimeria found, Eimeria tenella was the most commonly located species and least found species were Eimeria mitis and Eimeria praecox.*

Introduction

Broiler chicken, *Gallus gallus domesticus* are bred and raised specifically for their meats which are soft, pliable, smooth textured skin and flexible breast bone cartilage. They are one of the most common and widespread domestic animals. Among the different disease causing parasites of these birds, coccidians are one of the most important. They are known to affect poultry, resulting in great economic loss all over the world (**Braunius, 1980; Jadhav *et al.*, 2011**). It is caused mostly by the genus *Eimeria* which are of the Apicomplexan parasite group (**Shirley, 1995**). The disease is classified as intestinal coccidiosis when the small intestine is affected and caecal Coccidiosis when the large intestine (caeca) is affected (**Adhikari, *et al.*, 2008**). About 1800 *Eimeria* species affect the intestinal mucosa of different animals and birds (**Shirley, 1995**). The intensity of infection of *Eimeria* species differ both globally and locally (**Amer *et al.*, 2010**) and with time (**Haug *et al.*, 2008**). In a study by Ahmad et al. (2012) 21.24% of chickens examined were infected with five species of *Eimeria*. Young layer chickens have greater infection ratio as compared with adults (**Bachaya *et al.*, 2012**).

Coccidiosis is characterized by dysentery, enteritis, drooping wings, poor growth and low production. In all parts of the world including Manipur where confinement rearing is practiced, Coccidiosis represents a major disease problem demanding attention of poultry producers, feed manufacturer and poultry disease experts. It is considered to be a disease of poor management that encouraged oocyst sporulation, contamination of feeds, bad ventilation and high stocking density (**Ruff, 1993**). The present study is an attempt to investigate the species of *Eimeria* found in Manipur, their prevalence and intensity of infection.

Materials and Methods

Study area:

The study was carried out in valley districts Manipur such as Imphal East and West, Bishnupur district and Thoubal district between June 2011 to June 2012. Manipur is one of the states of North - East India. The state is bounded by Nagaland in the north, Mizoram in the south, Assam in the west, and by the borders of the country Burma in the east as well as in the south. The state lies at latitude 23^0 83′ N – 25^0 68′ N and longitude 93^0 03′ E – 94^0 78′ E. The total area covered by the state is 22, 327 Sq Km.

Sample collection:

Seven poultry commercial farms of broiler were visited for specimen collection during April, 2012 to April, 2013. A total of 100 faecal samples and fifty carcasses were examined. Fresh faecal droppings were collected in sterile universal bottles and carcasses were collected in polythene leather bags and transported to the laboratory immediately for processing. Laboratory examination by wet mount smears of the faecal droppings as described by **Fleck and Moody (1993)** have been done. Concentration technique was also used for counting of oocyst as described by **Brown (1983).**

Post mortem examination was carried out after opening of the carcasses, and the intestine was removed aseptically, checked for the presence of lesions, the intestine scraped and observed under the microscope as per the method described by **Jordan and Pattison (2002**).

Parasitic processing and identification:

At least 1g of fresh stool sample was suspended in a tube containing 10ml of 0.85% saline. The suspensions were sieved through an 80 mesh. The strained suspension was centrifuged at 700 g for 5 min. And the supernatant was then decanted. The supernatant was discarded and diagnostic methods were performed on precipitates from each tube.

Direct smear: the precipitates were examined microscopically following direct method:

In brief, direct microscopy of the smears in a saline (0.85% NaCl solution) and Lugol's iodine was performed for the detection of Trophozoites, cysts and ova of intestinal parasites.

Sporulation with potassium dichromate: the precipitates were used for coccidian sporulation. Sporulation was performed in wet chamber at 24-26°C in a 2.5% aqueous solution of potassium dichromate ($K_2Cr_2O_7$).

Modified Ziehl-Neelsen staining: Modified ZN staining (**Kinyoun's** modification of acid fast staining) was done on

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smears made from fresh samples. The slides were screened under 100 X objectives of light microscope for identification of the coccidian parasites (**Kuzehkanan, 2011**)

Result and discussion

The result shows the presence of five Eimeria species – *E. tenella, E. maxima, E. mitis, E. acervulina and E. preacox.* **Hadipour et al. (2011**) identified four *Eimeria species- E. tenella, E. acervulina, E. preacox* and *E. maxima* in native chickens in Iran. In an earlier work **Nematollahi et al. (2011**) reported the prevalence of five *Eimeria* species. In the hen, *Gallus gallus domesticus* L., nine species of *Eimeria* have so far been described from India (**Mandal, 1987**). **Franco, (1993); Karim and Begun, (1994); Thakari and Rai, (1996); Kiani *et al.*, (2007); Adhikari *et al.*, (2008)** also reported similar observations.

Descriptions:

***Eimeria maxima* (Fig. 1 a, b)**

The oocyst is ovoid, light yellowish-brown colour and slightly rough wall, measuring 21.43 – 42.84 µm by 16.32 – 30.6 µm (mean 23.15 µm by 32.44 µm) in size, and shape index is 1.4. A polar granule is present. The oocyst wall is 1.3 µm in thickness. A prominent steida body is present in sporocyst.

***Eimeria tenella* (Fig. 1 c, d)**

The oocyst is broad ovoid, yellowish in colour with a little difference between the two ends, measuring 14.28 µm – 31.62 µm by 9.18 µm – 26.52 µm (mean 22.64 µm by 16.93 µm) in size and shape index is 1.34. The oocyst wall is 1.02 µm thick.

***Eimeria mitis* (Fig. 1 e, f)**

The oocyst is spherical and smooth wall, measuring 10.2 – 21.42 µm by 9.18 – 20.4 µm (mean 16.63 µm by 18.05 µm) in size and the shape index is 1.09. A polar granule is present in sporulated oocyst without oocystic residuum. Micropyle is absent but steida body is present in sporocyst. Sporocystic residuum is centrally present.

***Eimeria acervulina* (Fig. 1 g, h)**

The oocyst is oval or ovoid, smooth walled and uniformly thick wall but anterior is slightly thin, measuring 17.34 – 22.44 by 14.28 – 21.42 (mean 16.78 by 20.00) in size and shape index is 1.19. Polar granule present but oocystic residual body is absent.

***Eimeria praecox* (Fig. 1 i, j)**

The oocyst is ovoid or more or less spherical in shape with yellowish in colour, measuring 17.34 – 24.48 µm by 13.26 – 19.38 µm (mean 15.09 µm by 19.38 µm) in size and shape index is 1.28. The oocyst wall is smooth with 0.9 µm thickness.

A total of 100 faecal samples and fifty carcasses were examined. Out of the 7 commercial farms, A, B, D, E and F have mud or mud with brick flooring while the rest of the farms C and G have concrete flooring. The total infection intensity was higher in mud or mud with brick flooring as indicated in table I. A total of 126 out of 150 samples of broiler chicken were found positive and predominance of *Eimeria* infection was 84% (126/150). The rate is higher as compared to other similar survey in different parts of the world **(Bachaya, et. al. 2012, Hadipour, et. al. 2011)**. Coccidial oocysts are omnipresent and are easily spread in the poultry house environment **(Bachaya et. al., 2012**). The lower prevalence of *Eimeria* in concrete type flooring might be due to frequent cleaning of floors and avoidance of pillage of litter. In mud or mud with brick type floored farms there is chance of the association of the *Eimeria* sp with the litter in cracks and crevices of the floor.

The study also revealed that all ages of poultry are susceptible to *Eimeria* infection but younger birds are more susceptible to infection as shown in Table 2. This is in agreement with the findings of **Omer *et al.* (2011)** and **Bachaya *et al.* (2012).** In the present study *E. tenalla and E. maxima* were found in all the farms surveyed so far at Manipur (Table. 3).

Among the 5 species of *Eimeria* surveyed the highest predominance was in the case of *E. tenella* while the least was in case of *E. mitis* and *E. praecox*. The highest prevalence of *E. tenella* might be due to its high pathogenicity and predominant nature. This is in agreement with the findings of **Adhikari *et al.* (2008). Beate and Martin (1999)** reported *E. acervulin, E. maxima and E. tenella* to be the most important protozoan parasites in poultry industry. The result of the present work also supports this view.

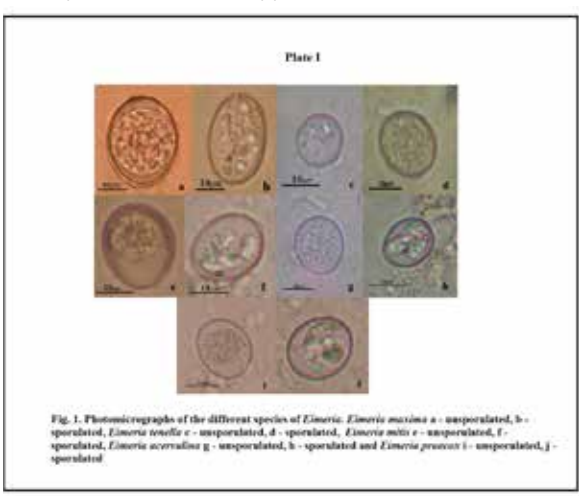

Fig. 1. Photomicrographs of the different species of *Eimeria*. *Eimeria maxima* a - unsporulated, b - sporulated, *Eimeria tenella* c - unsporulated, d - sporulated, *Eimeria mitis* e - unsporulated, f - sporulated, *Eimeria acervulina* g - unsporulated, h - sporulated and *Eimeria praecox* i - unsporulated, j - sporulated

Table1. Different *Eimeria* species identified and their measurements

Eimeria *identified*	Shape of the Oocyst	Size of Oocyst (Range) (µm)	Average (µm)	Shape Index	Thickness (µm)
E. tenella	Broad ovoid	14.28 – 31.62 x 9.18 –26.52	16.93x 22.64	1.34	1.32
E.maxima	Egg shape	14.28 – 42.84 x16.32 –30.60	23.15 x32.44	1.40	1.20
E. mitis	Spherical	10.20 – 21.42 x 9.18 – 20.40	16.63x18.05	1.09	1.02
E. acervulina	Ovoid	17.34 – 22.44 x14.28 –21.42	16.78x20.00	1.19	1.12
E. praecox	Ovoid	17.34 – 24.48 x13.26 –19.38	15.10x19.38	1.28	0.90

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Table 2: Prevalence of coccidian infection during 2011 to 2012 in different farms

Farms	Faecal droppings examined			Intensity of infection and percentage		Carcasses Examined			Intensity of infection and percentage	
	Total	Young	Adult	Young	Adult	Total	Young	Adult	Young	Adult
A	25	18	7	8 (44.4)	5 (71.4)	6	3	3	2(66.66)	1(33.3)
B	15	10	5	5 (50)	4 (80)	11	7	4	4(57.1)	2(50.00)
C	9	5	4	0 (0.00)	3 (75)	3	1	2	0(0.00)	1(50.00)
D	10	4	6	1 (16.66)	4 (66.66)	5	3	2	2(66.66)	0(0.00)
E	12	6	6	2 (33.3)	0 (0.00)	12	5	7	3(60.00)	5(71.4)
F	18	8	10	5 (62.5)	6 (60)	9	5	4	3(60.00)	2(50.00)
G	11	5	6	3 (60)	0 (0.00)	4	2	2	1(50)	0(0.00)
Total	100	53	47	24 (45.28)	22(46.80)	50	26	24	15(57.69)	11(45.83)

Young (2 - 4 weeks) and Adult (5 weeks and above)

Table 3: Different *Eimeria* species in different farms and their percentage of prevalence in 7 different farms.

Farm	E. tenella	E.maxima	E. mitis	E. acervulina	E. praecox
A	0(0.00)	1(16.67)	0(0.00)	2(33.3)	0(0.00)
B	3(27.3)	1(9.09)	0(0.00)	1(9.09)	1(9.09)
C	1(33.3)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
D	1(20.00)	1(10.00)	0(0.00)	0(0.00)	0(0.00)
E	2(16.7)	3(25.00)	1(8.33)	1(8.33)	1(8.33)
F	2(22.2)	1(11.1)	1(11.1)	0(0.00)	1(11.1)
G	1(25.00)	0(0.00)	0(0.00)	(0.00)	0(0.00)
Total	10	7	2	4	3

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