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Diversity of *Eimeria* species in backyard poultry of subtropical hilly region of Meghalaya, India

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Abstract
The aim of the present study was to determine the prevalence and diversity of *Eimeria* species in the backyard poultry of hilly region of Meghalaya. A total of 674 nos. of fecal samples of poultry were collected from different age groups *viz.* < 8 weeks (132 nos.), 8-28 weeks (290 nos.) and > 28 weeks (252 nos.) and examined for detection of *Eimeria* oocysts by flotation techniques. Sporulation of the oocyst was done in 2.5% potassium dichromate solution for identification of the *Eimeria* species. The overall prevalence of *Eimeria* sp. in the backyard poultry was 30.12%. Eight species of *Eimeria viz. E. tenella* (24.63%), *E. necatrix* (10.84%), *E. maxima* (0.98%), *E. mitis* (1.48%), *E. brunetti* (1.97%), *E. praecox* (1.48%), *E. mivati* (0.98%) and *E. acervulina* (2.96%) were recorded. Mixed infections were recorded in 54.68% birds. Highest and lowest infections was recorded in the month of May (40.29%) and December (15.15%), respectively. Season wise, highest infection recorded during monsoon (33.87%) followed by spring (32.77%), winter (27.78%) and autumn (18.37%). Age wise, 68.18%, 25.86% and 15.08% infections were recorded in < 8, 8-28 and > 28 weeks old birds, respectively.

Keywords: diversity, *Eimeria* sp., backyard poultry, Meghalaya

1. Introduction
Animal husbandry is an important subsector of Indian agriculture and backyard poultry farming is one of the important components among the tribal farmers of Meghalaya. Backyard poultry farming is increasing rapidly due to low establishment cost, cheap source of proteins and employment [1, 2]. The total poultry population of the country is 851.81 million (backyard poultry: 317.07 million; commercial poultry: 534.74 million), in which Meghalaya's contribution is 0.63% (5.38 million) [3].
Poultry coccidiosis is an intracellular intestinal parasitic disease caused by the different species of *Eimeria.* Morbidity of coccidiosis is estimated to be 50-70% and the disease is a major threat to 15-50 day old birds [4]. It seriously impairs the growth and feed utilization of the infected birds resulting in the loss of productivity and inflicts tremendous economic losses to the poultry farmers. It causes intestinal tissue damage and interferes with the food digestion and absorption resulting in the weight loss and bloody droppings. Sometime high rate of mortality may be observed in a farm. Secondary bacterial infection with *Clostridium perfringens* may occur which may predispose them to other gut infections such as necrotic enteritis. About nine species of *Eimeria* have been recognized in poultry birds, of which *E. brunetti*, *E. maxima*, *E. necatrix* and *E. tenella* are the most pathogenic; *E. acervulina*, *E. mitis*, *E. mivati* are the less pathogenic while *E. praecox* and *E. hagani* are the lesser pathogenic [5, 6]. The prevalence of coccidiosis in poultry was reported in many countries such as Iran [7], Egypt [8], Ethiopia [9], South Africa [10], Nepal [11], Korea [12] and Nigeria [13]. Both clinical and sub-clinical coccidiosis retards the growth of flocks and cause huge economic loss to the farmers [14], thus it is important to know the prevalence of different species of *Eimeria* in a particular geographical region. Though there are reports on the prevalence of different species of *Eimeria* in the poultry from different states of India like Assam [15], Uttar Pradesh and Uttarakhand [16], Jammu [17], Karnataka [18], Tamil Nadu [19] but there is paucity of information on different species of *Eimeria* in backyard poultry from Meghalaya. Thus, the present study has been undertaken to find out the diversity of *Eimeria* sp. in the backyard poultry of subtropical hilly region of Meghalaya.

2. Materials and Methods
2.1 Study area: The present study was conducted in the Ri-Bhoi district of Meghalaya which

lies between 25°15´ and 26°15´ North latitudes and 91°45´ and 92°15´ East longitudes. The district is characterized by rugged and irregular land surface and includes a series of hill ranges.

2.2 Study period: The study was conducted for two years (2018-19, 2019-20) and divided into four seasons, *viz.* Spring (March, April), Monsoon (May, June, July, August, September), Autumn (October, November) and Winter (December, January, February).

2.3 Sample collection: Freshly voided fecal samples were collected from different locations *viz*. Umiam, Sarikuchi, Umthan, Umsawkhwan, Mawphrew, Nalapara, Borkhatsari, Purangang, Borgang and Lalumpam in the marked plastic pouch/vials. All the birds were categorized according to the age *viz.* < 8 weeks, 8-28 weeks and >28 weeks. A total of 674 nos. of fecal samples of poultry were collected from different age groups *viz.* < 8 weeks (132 nos.), 8-28 weeks (290 nos.) and > 28 weeks (252 nos.).

2.4 Parasitological techniques: To detect *Eimeria* oocysts in the fecal samples of backyard poultry, samples were examined by direct flotation technique using saturated salt (sp.gr. 1.20) and sucrose (sp.gr. 1.27) solution [20]. Positive samples were then quantified to estimate the oocyst per gram (OPG) of feces by using modified McMaster technique [21]. Samples not being examined on the same day were preserved at refrigerated temperature (4°C) for next day examination. Sporulation of the oocyst was done by mixing positive samples having *Eimeria* oocysts with 2.5% potassium dichromate solution in a ratio of 1:5 volumes as per the standard procedure [21, 22]. Morphological characterization of the oocysts [20] was done by using an Olympus BX51 light microscope at 100x and 400x magnifications.

3. Results and Discussion

The overall prevalence of coccidiosis in backyard poultry of Meghalaya was 30.12% (Table 1). Eight species of *Eimeria viz. E. tenella* (24.63%), *E. necatrix* (10.84%), *E. maxima* (0.98%), *E. mitis* (1.48%), *E. brunetti* (1.97%), *E. praecox* (1.48%), *E. mivati* (0.98%) and *E. acervulina* (2.96%) were identified by morphological characterization (Fig. 1). Mixed infections were recorded in 54.68% birds. Month wise, highest and lowest infection was recorded in the month of May (40.29%) and December (15.15%), respectively (Fig. 2). Oocyst per gram (OPG) was recorded highest and lowest in the month of August (30000) and February (9500), respectively. Season wise, highest infection recorded during monsoon (33.87%) followed by spring (32.77%), winter (27.78%) and autumn (18.37%) (Table 1, Fig.3). *E. tenella*, *E. necatrix* and mixed infections were observed throughout the year (Fig. 2). However, in monsoon season *E. praecox*, *E. maxima*, *E. mitis*, *E. brunetti* and *E. acervulina* are also observed; *E. brunetti* in spring; *E. maxima*, *E. brunetti*, *E. praecox*, *E. mivati* and *E. acervulina* in winter; *E. mitis*, *E. praecox* and *E. acervulina* in autumn.

Table 1: Seasonal prevalence of *Eimeria* sp. in backyard poultry of hilly region of Meghalaya

Season	Sample examined	Sample positive	*E. tenella*	*E. necatrix*	*E. maxima*	*E. mitis*	*E. brunetti*	*E. praecox*	*E. mivati*	*E. acervulina*	Mixed Infection	OPG
Spring	119	39 (32.77)	10(25.64)	5 (12.82)	-	-	1 (2.56)	-	-	-	23 (58.97)	1162.50
Monsoon	313	106(33.87)	25(23.58)	12(11.32)	1(0.94)	2(1.89)	1 (0.94)	1 (0.94)	-	2 (1.89)	62 (58.49)	23750
Autumn	98	18 (18.37)	6 (33.33)	1 (5.56)	-	1(5.56)	-	1(5.56)	-	1(5.55)	8(44.44)	23250
Winter	144	40 (27.78)	9 (22.5)	4 (10)	1 (2.5)	-	2 (5)	1 (2.5)	2 (5)	3 (7.5)	18 (45)	11866.66
Total	674	203 (30.12)	50(24.63)	22(10.84)	2(0.98)	3(1.48)	4(1.97)	3(1.48)	2(0.98)	6(2.96)	111(54.68)	18550

Figures in parentheses indicates percent positivity

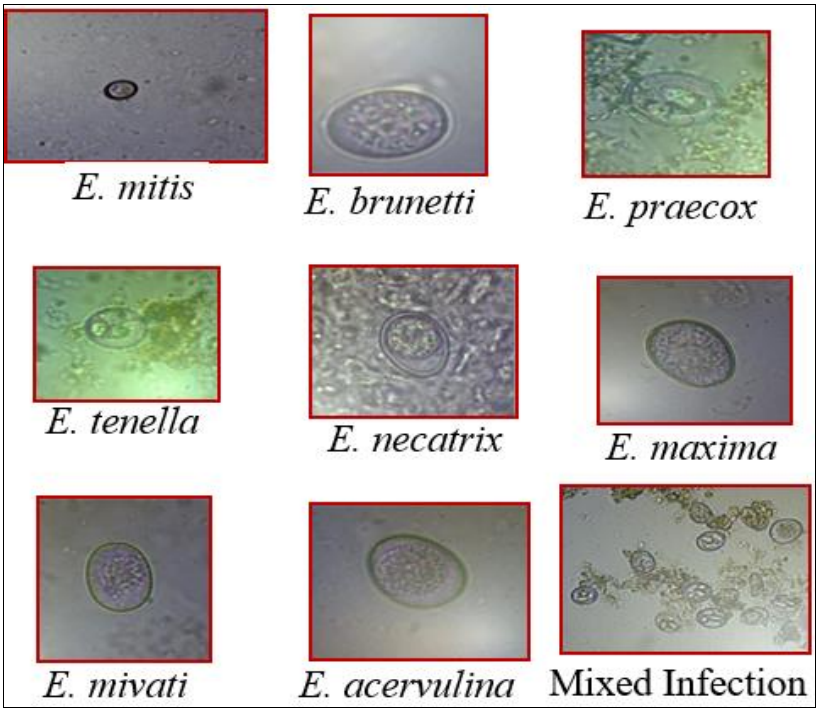

Fig 1: Different species of *Eimeria* in backyard poultry of Meghalaya

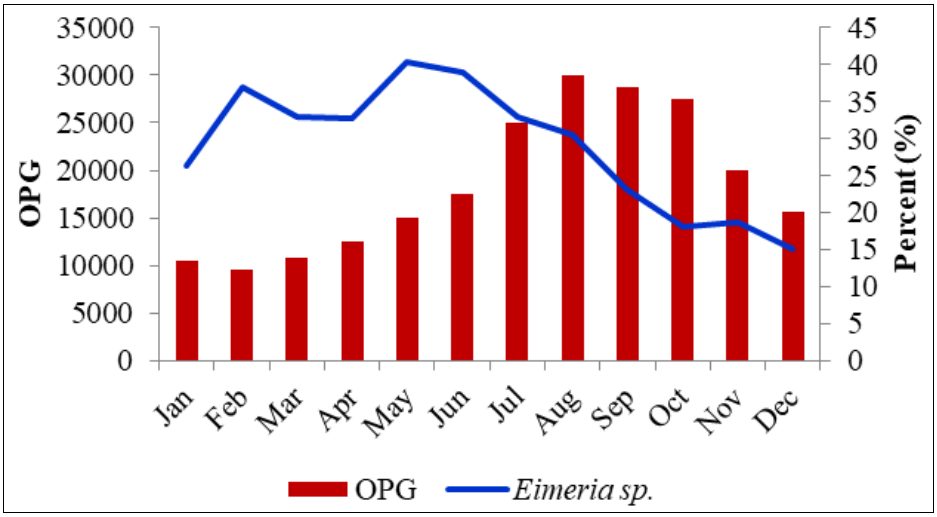

Fig 2: Month wise Intensity of *Eimeria* infection in poultry of Meghalaya

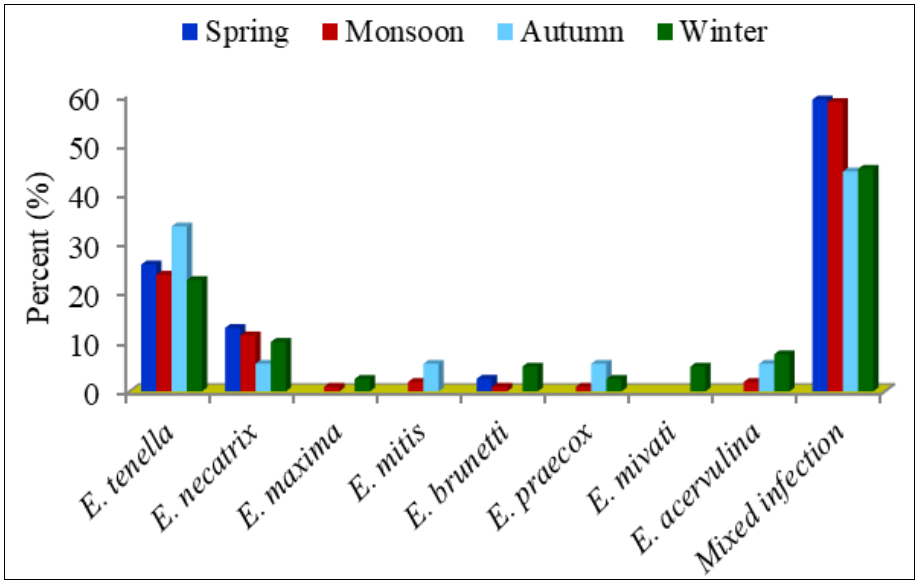

Fig 3: Season wise prevalence of different species of *Eimeria* infection in poultry

In the present study, different species of *Eimeria* in poultry has been observed which was in agreement with the findings of Adhikari *et al.* [11], Sharma *et al.* [17] and Grabi *et al.* [23]. Mixed infections of *Eimeria* sp. was observed in 54.68% birds. Kaboudi *et al.* [24] and Molla and Ali [25] reported 26.55% and 45.65% mixed infections in the poultry birds from Tunisia and Ethiopia, respectively. The difference in the percent prevalence from the present study may be due to different geographical locations.

In the present study, highest and lowest infections were recorded in the month of May and December, respectively. Similarly, Naveed and Faryal [14] reported highest numbers of cases in August (90.6%) and least in January (63%). They also observed that the type of litter materials, time duration, season, humidity, temperature and disposal of dead birds are other critical factors for poultry coccidiosis. High rate of infection in the monsoon season may be due to wet floor and litter of poultry farm which is very conducive for the growth and development of the *Eimeria* oocysts. *E. tenella*, *E. brunetti* and *E. necatrix* are associated with haemorrhagic coccidiosis and can be highly pathogenic, with high mortality and morbidity [26]. However, *E. acervulina*, *E. maxima*, *E. mitis* and *E. praecox* are less pathogenic, incurring malabsorptive pathologies, although morbidity and mortality can occur depending on dose ingested, parasite strain-specific variation in virulence and host factors such as age, breed and immune status [27]. According to the Williams *et al.*[28] co-infection with multiple species is common and can complicate diagnosis. *E. necatrix* has been recognized as the most pathogenic *Eimeria* sp. but *E. tenella* is more common and exerts a greater impact on poultry production [29]. The occurrence of coccidiosis can also vary due to climatic conditions, with evidence of elevated parasite levels and disease during wetter and warmer seasons [30,31,32].

Age wise 68.18%, 25.86% and 15.08% infections were recorded in < 8, 8-28 and > 28 weeks old birds, respectively (Table 2). In poultry birds of 8-28 weeks, all eight species of *Eimeria* were prevalent *viz. E. tenella* (21.33%), *E. necatrix* (14.67%), *E. maxima* (1.33%), *E. mitis* (4%), *E. brunetti* (5.33%), *E. praecox* (2.67%), *E. mivati* (2.67%) and *E. acervulina* (5.33%). In birds of < 8 weeks, *E. tenella* (31.11%), *E. necatrix* (8.89%), *E. maxima* (1.11%), *E. praecox* (1.11%) and *E. acervulina* (2.22%) were recorded. However, in birds of > 28 weeks only *E. tenella* (15.79%) and *E. necatrix* (7.89%) were recorded. Percent prevalence of mixed infections was 55.56%, 42.67% and 76.32% in < 8, 8-28 and > 28 weeks old birds, respectively.

Table 2: Age wise prevalence of *Eimeria* sp. in backyard poultry of hilly region of Meghalaya

Age group	Sample examined	Sample positive	*E. tenella*	*E. necatrix*	*E. acervulina*	*E. praecox*	*E. mivati*	*E. mitis*	*E. maxima*	*E. brunetti*	Mixed infection
< 8	132	90 (68.18)	28 (31.11)	8 (8.89)	2 (2.22)	1 (1.11)	-	-	1 (1.11)	-	50 (55.56)
8-28	290	75 (25.86)	16(21.33)	11(14.67)	4(5.33)	2(2.67)	2(2.67)	3(4)	1(1.33)	4(5.33)	32(42.67)
> 28	252	38 (15.08)	6 (15.79)	3 (7.89)	-	-	-	-	-	-	29 (76.32)
Total	674	203(30.12)	50(24.63)	22(10.84)	6(2.96)	3(1.48)	2(0.98)	3(1.48)	2(0.98)	4(1.97)	111(54.68)

Figures in parentheses indicates percent positivity

In the present study, age wise variation in the prevalence of *Eimeria* infection in backyard poultry was observed and younger groups are more susceptible to infection. This is in congruence with the findings of Jemimah *et al.* [13], Wondimu *et al*. [33], Prakashbabu *et al*. [34] and Lawal *et al.* [35]. Williams [36] also reported that young birds are more susceptible to *Eimeria* sp. According to Long *et al*. [37] and Williams [38], excretion of oocysts has also been reported to be higher in birds between 4 and 8 weeks of age.

According to Omer *et al.* [39], all ages of birds are susceptible to coccidiosis, but younger birds are more susceptible to infection than older birds which might be associated with the immature immune system in young birds leaving them susceptible to infection even with the lower or less pathogenic strain of *Eimeria* species. Das *et al*. [40] also observed that high rate of infection in young birds may be due to decreased immunity as well as continuous exposure to infections from the contaminated litter. According to Morris and Gasser [41], *Eimeria* sp. multiply in the intestinal tract, causing tissue damage, interruption in feeding and digestive processes as well as nutrient absorption, blood loss and increased susceptibility to other disease agents.

The combinations of different *Eimeria* species and the intensity of infection vary considerably, both locally and globally [42, 43]. Prakashbabu *et al.* [34] also observed that occurrence of *Eimeria* species varies between geographic regions and poultry production systems and may also influence its genetic diversity. High incidence of coccidiosis is usually observed in poultry managed under intensive management system like deep litter due to increased likelihood of high oocysts accumulation in the litters [44, 45]. Factors which contribute to the outbreaks of clinical coccidiosis include litter moisture exceeding 30%, immune suppression, suboptimal inclusion of anticoccidials in feed and environmental and managemental stress [46]. Thus, the present study has significance because eight species of *Eimeria* were recorded for the first time in the different age groups of backyard poultry in the subtropical hilly region of Meghalaya. So, to make poultry farming profitable in this region it is necessary to implement preventive and control measures strictly against poultry coccidiosis.

4. Conclusion

The present study revealed that eight species of *Eimeria* are prevalent in the subtropical hilly region of Meghalaya and mostly prevalent during the monsoon season. Younger age groups are highly susceptible to the infections. Regular screening and use of anticoccidial drug is necessary for profitable backyard poultry farming.

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