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Investigation of *Eimeria* Oocyst Concentrations and Species Composition in Litter from Commercial Broiler Farms

Mohammad Zahangir Alam[1]✉, Shirin Akter[1], Md. Tohidul Islam[1], Fahima Khatun[2], Shanaz Parvin[1], Anita Rani Dey[1]

[1]Department of Parasitology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

[2]Department of Pathobiology, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

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Correspondence
Mohammad Zahangir Alam
✉: mzalam@bau.edu.bd

ABSTRACT

Coccidiosis is one of the major economically important diseases of poultry worldwide. In Bangladesh, coccidiosis is one of the major threats for commercial poultry industry as well. Successful control of chicken coccidiosis in Bangladesh mainly depends on the application of anticoccidial drugs (ACD) in ration of chickens during grow-out. There are some reports on the occurrence of coccidiosis in broiler chickens in Bangladesh, but information on track changes in *Eimeria* oocysts levels and species composition in commercial broiler farms over time during ACD program is very scarce. The purpose of this study was to investigate the oocyst concentrations and species composition of *Eimeria* in litter from commercial broiler houses during ACD control programs. Litter samples were collected from a total of 22 different broiler farms during at least one complete grow-out cycle. Samples were collected at 0, 1, 2, 3 and 4 week (wk) of grow-out of the ACD cycle. We succeeded for isolation of *Eimeria* oocysts from the litter samples of 14 broiler farms, and coccidian species were identified by previously described morphological characteristics using standard shape index method under microscopy. *Eimeria* oocyst concentration in litter was found to be reached in peak levels at 3–4 wk of grow-out cycle. However, sudden peak level of oocysts of *Eimeria* spp in 8 farms indicated to develop some drug resistance. *E. tenella, E. necatrix* and *E. acervulina* were generally found to be to present in the most of the samples. Findings of this study will be helpful to develop an appropriate control strategy against chicken coccidiosis in Bangladesh.

Introduction

Coccidiosis is one of the most common and economically important diseases of poultry industry in the world. It is caused by an intracellular parasite of the genus *Eimeria* that are commonly referred to as coccidia with a complex life cycle. Coccidia in commercial broilers are often assumed to be ubiquitous (Stayer *et al.*, 1995; McDougald, 2003). In Bangladesh, commercial poultry farming is started practically as an emerging agribusiness since 1980s (Haque, 2001) and contributes 51% of total meat production of the country (Raha, 2007). Coccidiosis is considered as one of the major threats for commercial broiler farms in Bangladesh (Farooq *et al.*, 1999) which causes a great economic loss to the poultry industry due to mortality, reduced body weight and expenses related to preventive and therapeutic control. The broiler industry in particular relies on continuous in-feed prophylaxis with application of anticoccidial drugs.

The coccidian parasite usually multiplies intracellularly in the epithelial and subepithelial cells of host gut resulting enteritis (Gordon and Jordan, 1982). As a result of mild to severe exposure, birds usually show a marked decrease in food and water consumption, decreased weight gains as a result of the disruption of the intestinal mucosa where minimal absorption is taking place. On the basis of affecting organs, the disease is classified as intestinal coccidiosis affecting the small intestine and caecal coccidiosis affecting the large intestine (Caeca). Coccidiosis is characterized by dysentery, enteritis, emaciation, drooping wings, poor growth and low production. This disease is common in all parts of the world where confinement rearing with poor management is practiced (Donal and McKenzie, 2007). Ultimately, the economic importance of the disease is due to its high morbidity and mortality in young birds, reduced feed conversion efficiency in sub-clinical cases in commercial farms. Therefore, coccidiosis represents a

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major problem in the poultry industry that grows the attention of the poultry producers, feed manufacturers and poultry disease experts.

In Bangladesh, a preliminary report on the presence of *E. tenella, E. necatrix* and *E. maxima* was confirmed by the fecal examination of chicks from Bangladesh Agricultural University Poultry Farm (Mondal and Qadir, 1978). In another earlier studies, seven *Eimeria* species had been reported in the local chickens of Bangladesh (Karim and Begum, 1994, Siddiki *et al*., 2014). The extent of the economic loss regarding certain coccidian species in Bangladesh is not clearly known because limited works have been conducted by concerning the topographical distribution of coccidian species in broiler chicken. A few reports on the chicken coccidiosis in commercial broiler farms have been documented in Bangladesh (Sharif *et al*., 2003; Iqbal *et al*., 2010, Iqbal *et al*., 2017).

Jordan and Pattison (1996) reported that at least nine species of *Eimeria* infect in poultry. Several authors have examined the composition of *Eimeria* species in poultry house litter under ACD programs (Chapman *et al*., 2016, Haug *et al*, 2008, Jenkins *et al.*, 2017). Information about the *Eimeria* oocysts concentration in poultry litter under ACD or live *Eimeria* oocyst vaccine (VAC) control programs is very important for successful control, prevention and eradication of avian coccidiosis. In this context mentioned above, the present study was attempted to investigate the concentration of *Eimeria* oocysts and to identify the different *Eimeria* species circulating in litter of commercial broiler farms in Bangladesh. This information may provide a means for assessing the efficacy of a particular control program and help to determine if switching between different anticoccidial compounds or to vaccination is warranted.

Materials and Methods

Selection of broiler farms

A total of 22 broiler farms were selected randomly from Mymensingh sadar and Muktagacha upazila of Mymensingh distrcit and Ghatail upazila of Tangail district for the collection of litter samples.

Collection of litter samples from the broiler farms

Litter samples were collected during at least one complete grow-out cycle. Samples were collected at 0, 1, 2, 3 and 4 wks of a broiler batch. 15-20 litter samples were randomly taken from the feeder and water line areas of each broiler farm.

Processing of samples

Collected litter samples were processed according to the method described by Jenkins *et al.* (2017). The litter sample slurries were transferred to a Waring blender. The sampling bottle was rinsed with another 250 ml tap water, which was then combined with the original sample, and the entire 500-ml slurry was mixed thoroughly in a Waring blender for 30 sec. A 25-ml aliquot of the slurry was transferred to a 50-ml polypropylene test tube containing 25 ml sucrose solution followed by vigorous mixing for 15 sec.

Determination of concentration of Eimeria spp.

Oocyst concentration (OPG-oocysts per gram of litter) was determined using a standard McMaster technique (Haug *et al*., 2008). In this technique, a special type of slide (McMaster slide) and as flotation fluid, saturated salt solution (sp.gr, 1.120) were used to separate the oocysts from the fecal material in a counting chamber of McMaster slide.

Ioslation and sporulation of oocysts

For isolation of oocysts, a 25-ml aliquot of the slurry was transferred to a 50-ml polypropylene test tube containing 25 ml sucrose solution followed by centrifugation at 2,500 X *g* for 5 min at 4^0C. *Eimeria* oocysts floating at the sucrose-water interface were removed using a 1-ml pipettor and transferred to a clean, 15-ml polypropylene test tube, mixed with water, and centrifuged at 2,500 X *g* for 10 min at 4^0C. Concentrated oocysts were preserved in 2% potassium dichromate at 28 ^{o}C for sporulation. After sporulation, potassium dichromate was removed by repeated centrifugation and resuspension in water.

Microscopic identification of Eimeria spp.

For identification of *Eimeria* spp., litter samples from 14 farms were processed for isolation of oocysts and morphological characteristics of the oocysts was identified using the ocular micrometer (Figure 1) followed by calculation of dimensions (length and width) and shape index according to Urquhart *et al.*, 1996 and Amer *et al.*, 2010 (Table 1).

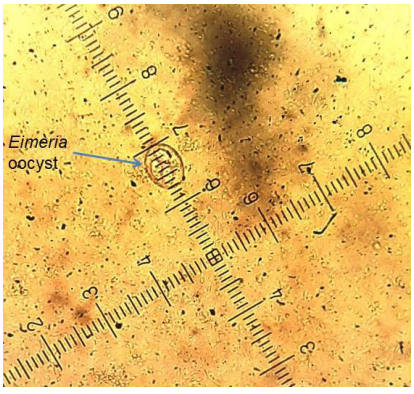

Figure 1. Measurement of an *Eimeria* oocyst using calibration by ocular micrometer

Table 1. Morphological characteristics of different *Eimeria* oocyst isolates (Urquhart *et al.*, 1996; Amer *et al.*, 2010).

Eimeria sp.	Oocyst size	Shape index	Shape
E. acervulina	18 x 14µm	1.28	Ovoid
E. brunetti	25 x 19µm	1.31	Ovoid
E. maxima	30 x 20µm	1.50	Ovoid
E. mitis	16 x 15µm	1.06	Sub-spherical
E. necatrix	20 x 17µm	1.17	Ovoid
E. praecox	21 x 17µm	1.23	ovoid
E. tenella	23 x 19µm	1.21	Ovoid

Results and Discussion

Eimeria oocysts concentration in litter

The concentration of *Eimeria* oocysts in litter at different broiler farms were shown in Table 2 and Figure 2. In this study, it was found that *Eimeria* oocyst concentration in litter reached peak levels at 3–4 week of grow-out cycle in almost all the 22 broiler farms. However, there was a sudden peak in oocysts number in 8 farms in between 3-4 weeks (Farm no. 2, 5, 6, 7, 10, 13, 16, 20) as shown in Table 2. Similarly, it was shown in another report that under a typical ACD program, *Eimeria* oocyst numbers in litter gradually increase until 4 wk after the placement of chicks (Jenkins *et al.*, 2017). It was also observed that *Eimeria* oocysts generally peak at about 2 wk under good VAC program because vaccinated chicks begin cycling oocysts earlier than when under an ACD program (Jenkins *et al.*, 2017). Sudden peak in oocysts numbers in many farms found in this study usually indicate some level of *Eimeria* spp. drug resistance or management problem complicating ACD programs. The gradual increase is believed to derive from a small number of oocysts that escape the effects of anticoccidial compounds and ACD resistance would be expected to lengthen the interval of oocyst increase through 5–6 wk of grow-out (Jenkins *et al.*, 2017). The abundance of *Eimeria* oocyst may be related with several factors such as the type and duration of ACD being employed, moisture content of poultry litter (Chapman *et al*., 2004, Chapman *et al.*, 1992) and initial concentration of oocysts at the time of placement (Graat *et al*., 1996). Previously, it was reported that the greater concentration of oocyst could be attributed to poor hygienic conditions of the brooder house and the leaking troughs making the whole litter wet (Majaro, 1983). An emerging drug tolerance may happen due to the use of single ACD compound throughout the broiler's life. The advantage of measuring *Eimeria* oocyst concentrations in litter is that it provides insight into the efficacy of each ACD or VAC program in particular broiler farms (Jenkins *et al.*, 2017). Moreover, by examining changes in *Eimeria* oocyst levels over time of grow-out, and comparing this to patterns expected during a VAC or ACD program, one may gain insight into drug resistance or incomplete vaccine delivery.

Identification of Eimeria spp. in different broiler farms

In the present study, seven different species of *Eimeria* were identified viz. *E. tenella*, *E. necatrix E. acervulina, E. brunetti, E. praecox, E. acervulina and E. maxima* (Table 3, Figure 3) by examining the litter samples under microscope. Table 3 shows that *E. tenella, E. necatrix* and *E. acervulina* were generally present in the majority of the farms. Previously, on the basis of biological characteristics such as lesion, location and morphological characteristics, five species of *Eimeria* viz. *E. tenella, E. acervulina, E. necatrix, E. maxima,* and *E. brunetti* were identified in broiler chickens of Bangladesh and the highest prevalence (42%) was found in the form of mixed infection by *E. tenella* and *E. acervulina*. *E. tenella* (Iqbal *et al*., 2017). Adhikari *et al.,* (2008) and Franco (1993) also identified five species of *Eimeria* viz. *E. tenella, E. acervulina, E. necatrix, E. maxima* and *E. brunetti* in layer chickens of Nepal and Brazil, respectively. In another earlier studies, *Eimeria* species except *E. necatrix* have been reported in the local chickens of eastern hills of Nepal and also in Bangladesh (Karim and Begum, 1994, Thakuri and Rai, 1996). The morphological features especially the dimensions and shape index of the coccidian species were considered to determine the species identity. The shape index was often quoted as criteria for species identification but less variation was found in shape index of different species (Adhikari *et al.*, 2008).

In this study, *Eimeria* infection was mainly found as a mixed infection in the commercial broiler farms. The present findings are consistent with the previous finding reported in layer chickens of Nepal where the author found mixed *Eimeria* infection (50%) and monococcidian infection (25%) in layer chickens Adhikari *et al.* (2008). Our results also corroborate with the findings of Aryal (2001) and Gari *et al.* (2008) where they reported the avian coccidiosis occurring in the form of both mixed and mono infection of *E. tenella.*

The highest prevalence of *E. tenella* might be due to its highly pathogenic and predominant in nature and the highest prevalence of mixed infection might be found due to opportunistic nature of the mild pathogenic species of *Eimeria* which starts infection in the birds under sufficient stress due to initial infection with pathogenic species (Levine, 1942). There are several factors like species, management, seasons and topographical location of the farms which may influence the prevalence of mono and mixed infection of coccidiosis in broiler farms.

Table 2. Concentration of *Eimeria* oocysts in litter samples from different broiler farms

Site	Number of oocysts per gram of litter				
	0 WK	1 WK	2 WK	3 WK	4 WK
Farm 1 (Fulbaria)	0	67	133	333	366
Farm 2 (Digarkanda)	0	100	202	833	3500
Farm 3 (Shambhuganj)	0	133	400	550	666
Farm 4 (Shambhuganj)	0	0	166	233	400
Farm 5 (Shambhuganj)	66	100	333	6666	7666
Farm 6 (Shambhuganj)	0	166	1000	16000	15000
Farm 7 (Shambhuganj)	100	200	466	3466	40000
Farm 8 (Muktagacha)	0	66	200	366	500
Farm 9 (Muktagacha)	0	66	200	366	733
Farm 10 (Muktagacha)	0	0	133	2666	10767
Farm 11 (Muktagacha)	0	67	200	367	566
Farm 12 (Muktagacha)	0	0	167	233	533
Farm 13 (Muktagacha)	0	100	400	2067	21967
Farm 14 (Muktagacha)	0	0	100	333	433
Farm 15 (Ghatail, Tangail)	0	0	133	566	733
Farm 16 (Ghatail, Tangail)	66	366	1500	35000	40000
Farm 17 (Ghatail, Tangail)	0	0	700	1166	1400
Farm 18 (Ghatail, Tangail)	0	0	100	266	366
Farm 19 (Ghatail, Tangail)	0	133	600	733	800
Farm 20 (Ghatail, Tangail)	0	100	1533	13500	13333
Farm 21 (Ghatail, Tangail)	0	0	300	866	833

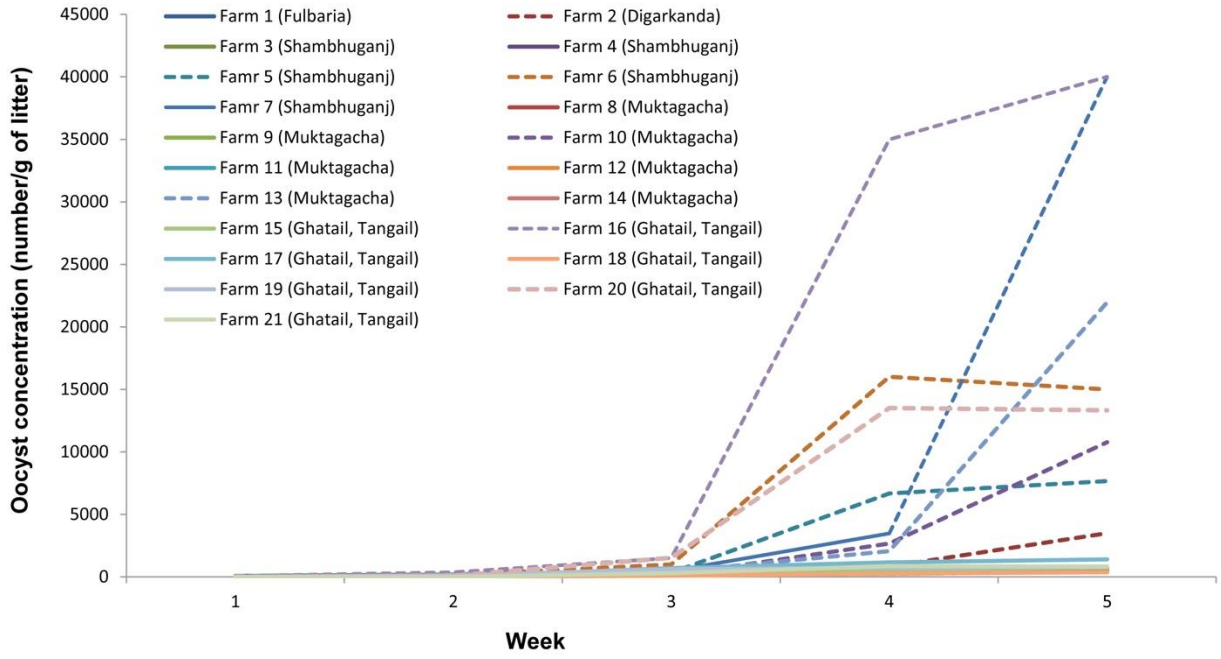

Figure 2. Concentration of *Eimeria* oocysts in litter samples from different broiler farms

Table 3. Identification of different *Eimeria* spp. in broiler farms

Site	*Eimeria* spp.						
	E. tenella	*E. necatrix*	*E. acervulina*	*E. brunetti*	*E. praecox*	*E. maxima*	*E. mitis*
Farm 1 (Fulbaria)	√	√			√		
Farm 2 (Digarkanda)	√	√	√				√
Farm 3 (Shambhuganj)	√		√			√	
Farm 4 (Shambhuganj)	√	√	√		√		
Farm 5 (Shambhuganj)		√	√				√
Farm 6 (Shambhuganj)		√	√				
Farm 7 (Shambhuganj)		√	√			√	√
Farm 8 (Muktagacha)		√	√	√	√		√
Farm 10 (Multagacha)	√	√	√	√			
Farm 11 (Muktagacha)	√	√	√	√			
Farm 14 (Muktagacha)	√	√	√	√	√	√	
Farm 17 (Ghatail, Tangail)	√		√	√	√		√
Farm 21 (Ghatail, Tangail)	√			√	√		
Farm 22 (BAU Farm)*	√		√		√		√

*Litter samples from Farm no. 22 (BAU Farm) were examined for the identification of *Eimeria* spp. but not for the determination of the oocyst concentration

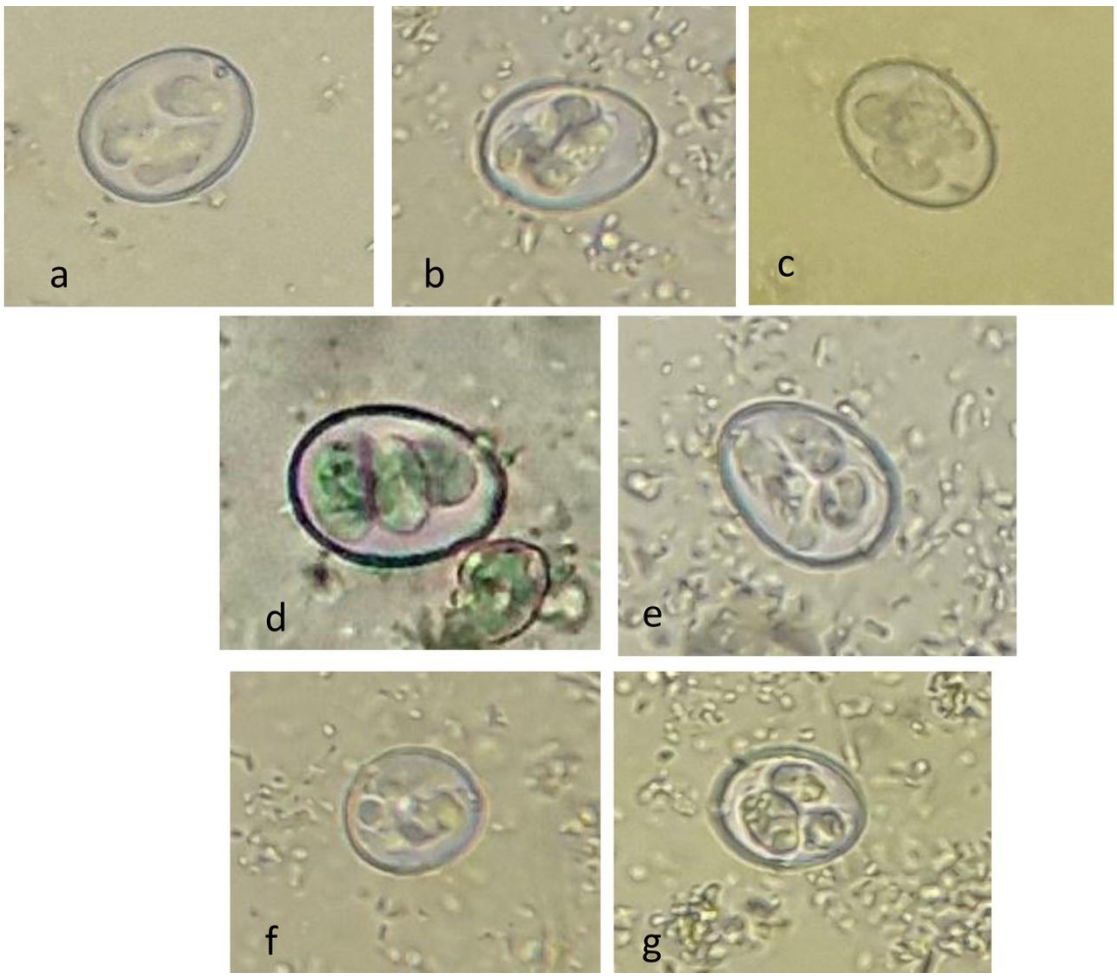

Figure 3. Oocysts of *Eimeria* spp. identified in litter samples collected from different broiler farms (a: *E. tenella*; b: *E. necatrix*; c: *E. praecox*; d: *E. maxima*; e: *E. brunetti*; f: *E. mitis*; g: *E. acervulina*)

Conclusions

Coccidiosis is one of the most important disease threats for commercial broiler production in Bangladesh and its treatment and control measures should have prime importance on the basis of species variation depending on their predilection sites. Investigation of *Eimeria* oocyst concentration and species composition in litter under ACD programs is essential for strengthening particular control program against coccidiosis. Moreover, identification of different species of *Eimeria* is important for prevention, surveillance, and control of coccidiosis in broiler farms of Bangladesh.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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