

RESEARCH ARTICLE

Molecular detection and risk factors of *Eimeria* in native and exotic chickens under varying management systems in Bangladesh

Md. Afazur Rahman¹, A R M Beni Amin¹, Kazi Farah Tasfia¹, Makoto Matsubayashi², Md. Shahiduzzaman^{1*}

1 Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh, **2** Graduate School of Veterinary Medical Sciences, Osaka Metropolitan University, Osaka, Japan

* szaman@bau.edu.bd



OPEN ACCESS

Citation: Rahman MA, Amin ARMB, Tasfia KF, Matsubayashi M, Shahiduzzaman M (2025) Molecular detection and risk factors of *Eimeria* in native and exotic chickens under varying management systems in Bangladesh. PLoS One 20(7): e0327037. <https://doi.org/10.1371/journal.pone.0327037>

Editor: Shawky M Aboelhadid, Beni Suef University Faculty of Veterinary Medicine, EGYPT

Received: April 11, 2025

Accepted: June 9, 2025

Published: July 15, 2025

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0327037>

Copyright: © 2025 Rahman et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/),

Abstract

A cross-sectional study was conducted in Bangladesh to determine the prevalence, molecular detection, and risk factors of *Eimeria* spp. infection in native and exotic chickens under various management systems. A total of 1,500 fecal samples were collected from different breeds, age groups, and sexes across multiple districts. Fecal examination using flotation and McMaster techniques identified positive cases, followed by molecular detection of *Eimeria* species. A questionnaire survey was also conducted to assess potential risk factors. Among the 1,500 chickens, 87 (5.8%) were positive for *Eimeria* oocysts, with higher prevalence in exotic breeds (7.96%) than native breeds (4.13%). The prevalence rates were 18.40%, 13.98%, 12.09%, and 3.40% in Aseel, Broiler, Sonali, and Deshi chickens, respectively, with no infection found in Naked Neck, Hilly, or Fayoumi breeds. Molecular analysis detected six *Eimeria* species: *E. tenella* was detected in 64 samples (62.07%) and in all breeds with the highest occurrence in Aseel. *E. acervulina* was the second most common species (25.28%), found in 23 samples from Deshi, Broiler and Sonali breeds. Other species, including *E. brunetti*, *E. mitis*, *E. necatrix*, and *E. maxima*, were rare and sparsely distributed. Chickens fed commercial feed (7.88%) had significantly higher infection rates ($p < 0.0013$) than those on local feed (3.99%). Intensive rearing systems (15.27%) showed higher infection rates compared to free-ranging systems, but no infection occurred in intensive systems without litter or semi-intensive systems. This is the first comprehensive report on infection status of *Eimeria* in chickens including all native breeds rearing in different management system in Bangladesh.

Introduction

The poultry industry in Bangladesh has experienced rapid growth over the past few decades, transitioning from traditional backyard farming to more intensive and

which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data availability statement: All relevant data are within the manuscript.

Funding: This study was funded by the Bangladesh Academy of Sciences under the BAS-USDA Program (BAS-USDA Program/2023/26(12) to M.S.). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

commercialized systems. This expansion has significantly contributed to economic growth, job creation, and the availability of affordable protein sources [1]. According to the Bangladesh Poultry Industries Central Council (BPICC), the country produces approximately 1.5 million metric tons of chicken meat and over 10 billion eggs annually [2]. However, this growth has been accompanied by an increased prevalence of poultry diseases, particularly coccidiosis, which poses a significant challenge to both commercial and backyard farming systems.

Coccidiosis, caused by protozoan parasites of the genus *Eimeria*, is one of the major diseases affecting poultry worldwide. The warm and humid climate of Bangladesh, combined with suboptimal management practices and inadequate biosecurity measures, creates favorable conditions for *Eimeria* proliferation. The disease leads to considerable economic losses due to increased mortality, reduced body weight, and expenses associated with preventive and therapeutic control measures [3].

Several factors contribute to the high prevalence of coccidiosis in Bangladesh, including poor housing conditions, overcrowding, improper litter management, and unregulated use of anticoccidial drugs. Additionally, non-scientific rearing practices, such as mixing old and new litter and maintaining short rest periods between flocks (8–14 days), further increase disease transmission risks [4,5]. Poor biosecurity practices, such as farm visitors, live bird markets, and off-site worker accommodations, also elevate infection risks [6].

Poultry farming in Bangladesh operates under two primary systems: family-based (traditional backyard) farming and commercial production (small, medium, and large-scale farms) [7,8]. Backyard poultry farming primarily involves indigenous breeds such as Naked Neck, Deshi, Aseel, and Hilly chickens, which play a crucial role in rural livelihoods by providing food security and supplemental income. These low-input systems are integrated into household activities, relying on scavenging with minimal external feed supplementation. In contrast, commercial farms focus on high-yielding breeds such as Cobb 500 broilers and specialized layers. Sonali chickens, a cross-breed of Rhode Island Red and Fayoumi, are particularly popular for their adaptability and superior meat quality [9]. However, small and medium-sized farms often face challenges such as dependence on middlemen, inadequate disease management knowledge, and misuse of antibiotics and anticoccidials [10,11].

Accurate identification of *Eimeria* species is crucial for effective disease management. Traditional microscopic methods often fail to distinguish between different species, leading to diagnostic inaccuracies. Molecular techniques, such as PCR-based identification, provide higher sensitivity and specificity, enabling precise characterization of *Eimeria* species affecting poultry [12–14].

While previous studies have investigated coccidiosis in commercial broilers and Sonali chickens [5,9,15–17], there is limited data on indigenous breeds. Additionally, existing research has primarily focused on specific regions, leaving gaps in understanding the disease's prevalence across diverse agro-ecological zones of Bangladesh. The influence of different rearing systems (backyard, semi-intensive, and commercial) on coccidiosis prevalence and severity remains underexplored. A

comprehensive risk analysis across various chicken breeds and management practices is essential for sustainable and cost-effective poultry production.

This study aims to provide a comprehensive assessment of *Eimeria* infections in native and exotic chickens under different management systems in Bangladesh. The specific objectives are:

1. To determine the prevalence of *Eimeria* infections in different breeds of chickens across various poultry farming systems.
2. To molecularly characterize *Eimeria* species using PCR-based techniques for accurate identification.
3. To analyze the key risk factors associated with coccidiosis prevalence, including feeds, rearing systems, and breeds.

The findings of this study will provide valuable insights for poultry farmers, veterinarians, and policymakers to enhance disease management practices. By identifying high-risk farming systems and chicken breeds, the study will contribute to the development of targeted interventions, ultimately improving the productivity and sustainability of the poultry industry in Bangladesh.

Materials and methods

Description of study area and samples

This study was conducted across six major divisions of Bangladesh: Mymensingh, Rangpur, Dhaka, Shariatpur, Comilla, Rajshahi, Bogura, Narsingdi, and Chattogram (Table 1). A total of 1,500 fecal samples were collected from these regions to assess the prevalence of chicken coccidiosis and the associated risk factors. This comprehensive geographical coverage ensures a representative understanding of the prevalence of coccidiosis across different poultry farming systems in Bangladesh. Samples were collected from both exotic breeds (Sonali, Broiler, Fayoumi) and native breeds (Deshi, Aseel, Naked Neck and Hilly) of chicken.

Current anticoccidial practices in Bangladesh

Commercial farms raising both exotic and native chickens commonly use anticoccidial feed purchased from various companies, except for chickens reared in household free-range systems. These commercial feeds typically include anticoccidials, antibiotics, and growth promoters. The current anticoccidial practices in Bangladesh involve a rotational shuttle program combining ionophore and non-ionophore chemicals. The strategy includes the use of combinations such as maduramycin with nicarbazine, salinomycin with nicarbazine, monensin with nicarbazine, and sanduramycin with nicarbazine in alternating months (3 months each combination). Additionally, Decoquinate is used for one month, followed by Ethopabate for one month, and Clopidol for another month, completing a year-long shuttle program to manage coccidiosis effectively (Personal communication with company and farmers).

Fecal sample collection and oocysts observation

A total of 1,500 fecal samples were collected from 77 farms or flocks across nine districts in six major divisions of Bangladesh. From each farm or household, approximately 15–25 fecal samples were collected depending on flock size. Fresh fecal samples (approximately 7–10 grams) were collected by using individual wood stick from freshly voided feces randomly from the flock. Each fecal sample was placed in a plastic container and marked. To maintain diagnostic resolution, each sample was kept and processed individually; no pooling was performed. The age (young and adult), breed, sex, rearing system, and feed were noted against the corresponding marking of the samples (Table 2). Samples from male or female bird were collected upon identification of sex. The samples were then transported through icebox to the laboratory at the Department of Parasitology, Bangladesh Agricultural University for faecal examination. Samples were stored in the refrigerator at 4°C if not immediately examined.

Table 1. The specific locations within each region and their respective geographical coordinates of sample collection areas.

Locality/region	Longitude and latitude	No of farms/flocks (Total chicken)	Samples (N)/ examined birds
Mymensingh	Sadar (Town): 24°43'42.08"N, 90°24'28.86"E Shomvugang: 24°45'44.71"N, 90°26'53.12"E Vabokhali: 24°40'36.25"N, 90°26'50.54"E Trishal: 24°35'6.15"N, 90°23'17.20"E Chor: 24°43'52.31"N, 90°27'25.10"E Sutiakhali: 24°41'29.74"N, 90°26'51.65"E Kalir Bazar: 24°37'55.84"N, 90°27'50.22"E Nalitabari: 25° 5'5.23"N, 90°13'11.68"E	17 (6500)	478
Dhaka	BLRI (Savar): 23°53'19.31"N, 90°16'26.58"E Savar: 23°53'52.40"N, 90°15'49.36"E Rajeer: 23°12'23.27"N, 90° 3'7.40"E Madaripur: 23° 9'44.83"N, 90°12'12.27"E	10 (2270)	115
Comilla	Sarail: 24° 4'19.23"N, 91° 6'55.02"E Muradnagar: 23°39'34.83"N, 90°54'23.81"E Debidwar: 23°36'43.73"N, 90°58'0.31"E Burichang: 23°33'7.59"N, 91° 7'10.08"E Laksham: 22°57'5.12"N, 90°52'4.03"E Titas: 23°35'45.08"N, 90°50'14.18"E	9 (2160)	267
Shariatpur	Sadar (Town): 23°23'94N, 90°37'07E Amtoli: 23°18'72N, 90°35'82E	4 (1500)	95
Rangpur	Gangachara: 25°50'27.12"N, 89°13'45.70"E Haragach: 25°49'11.15"N, 89°19'46.66"E Mithapukur: 25°34'38.39"N, 89°16'50.97"E	6 (1650)	155
Rajshahi	Puba: 24°25'26.40"N, 88°39'26.99"E Bhangura: 24°12'28.98"N, 89°21'57.59"E	4 (250)	85
Bogura	Shahbandegi: 24°38'49.74"N, 89°25'9.97"E Town Kaleni: 24°50'0.22"N, 89°22'25.14"E	8 (730)	95
Narshingdi	Birpur: 23°55'44.48"N, 90°44'7.76"E Shivpur: 24° 1'43.78"N, 90°43'43.95"E	6 (240)	72
Chattogram	Alikadam Bazar: 21°38'44.37"N, 92°18'26.68"E Matiranga Bazar: 23° 2'33.43"N, 91°52'19.97"E Marma Borokholo: 22°23'31.92"N, 92° 3'12.85"E	13 (860)	138
Total		77 (16160)	1500

Note: At least 10 birds were examined from each farm/flock

<https://doi.org/10.1371/journal.pone.0327037.t001>

At the day of examination, the sample of an individual chicken was blended by a mortar and pistol, and then the floatation technique [18] was applied using saturated sodium chloride solution to concentrate oocysts aiming oocyst screening (oocysts per gram-OPG) and downward application. Five grams of homogenized droppings were mixed with 45 ml NaCl saturated salt solution (density = 1.20 g/ml) and filtered to remove coarse particles. Processed solution was poured through a tea strainer into a beaker then into a 15 ml centrifuge tube. For quantification, the McMaster counting technique as outlined by Hodgson [18] was used. A 0.3 ml aliquot of the fecal suspension was loaded into a McMaster counting chamber, filling both grids (0.15 ml per grid). Later the centrifuge tube was covered with a cover slip and allowed to stand for 20 minutes and then removed and placed on a slide and examined at 10x and then 40x magnifications to identify the oocyst [19].

Molecular detection of *Eimeria* species

To determine the species of *Eimeria* prevailing in different chicken farms of Bangladesh, the microscopically positive samples were subjected to DNA extraction and PCR. DNA was extracted using DNAzol Reagent (Invitrogen, USA) according

Table 2. Prevalence of *Eimeria* infection based on chicken-related risk factor.

Risk factors	Categories	No of samples examined	No. of positive samples (%)	Average OPG (range)	No of farms/flocks (coccidiostat used)	No of positive farms/flock (%)	Significance (p<0.05)
Exotic Breed	Sonali	182	22 (12.09)	133–1066	12(12)	8 (66.67)	*
	Broiler	186	26 (13.98)	33–1967	13(13)	10 (76.92)	*
	Fayoumi	235	0	0	5(3)	0	
	Subtotal	603	48 (7.96)				
Native Breed	Deshi	382	13 (3.40)	33–133	18(1)	7 (38.89)	
	Aseel	125	23 (18.40)	66–500	5(5)	5 (100)	*
	Naked Neck	227	0		15 (1)	0	
	Hilly	138	0		5 (0)	0	
	Subtotal	872	36 (4.13)				
Mixed population	Mixed (Sonali+Deshi)	15	1 (Sonali, 6.67)	66–100	2(1)	1 (50.00)	
	Mixed (broiler+Deshi)	10	2 (Broiler, 20.0)	33–133	2(2)	1 (50.00)	*
	Subtotal	25	3 (12.0)				
Sex	Male	255	25 (9.80)				*
	Female	1245	62 (4.98)				
Age	Adult (>60 days)	334	2 (0.17)				
	Young (1–60 days)	1166	85 (25.45)				*
Rearing system	Intensive with litter	478	73 (15.27)		25 (20)	17 (68)	*
	Intensive without litter	172	0		7 (5)	3 (42.86)	
	Semi-intensive with litter	50	0		10 (8)	3 (30.00)	
	Semi-intensive without litter	154	0		5 (3)	2 (40.00)	
	Free range	646	14 (2.17)		30 (2)	7 (23.33)	
Feed	Commercial feed	698	55 (7.88)		49 (36)	27 (55.10)	*
	Local feed	802	32 (3.99)		28 (2)	5 (7.14)	

Note: Out of 1,500 samples collected from 77 farms/flocks, 87 tested positive for coccidiosis, with 32 farms reporting positive cases and 38 farms using commercial feed containing coccidiostats.

Risk factor categories marked with * show statistically significant differences (p<0.05) in prevalence based on Chi-square test.

<https://doi.org/10.1371/journal.pone.0327037.t002>

to the manufacturer instructions with initial breaking of oocyst wall by using a Bead beater. Extracted DNA samples were tested by PCR using the *Eimeria* species specific primers (Table 3, Fig 1).

All primers used in this study were synthesized by Macrogen Inc., Korea, and were MOPC-purified to ensure high specificity and quality for PCR amplification. PCR reactions were performed in a final volume of 25 µL, containing 12.5 µL of 2 × PCR Master Mix (Takara, Japan), 0.5 µM of each forward and reverse primer, 2 µL of template DNA, and nuclease-free water to adjust the final volume. Positive controls included DNA extracted from field isolated *Eimeria* species confirmed by sequence analysis. Negative controls consisted of PCR reactions with nuclease-free water in place of template DNA. These controls were included during PCR run to validate the amplification results and rule out contamination.

PCR was performed in miniPCR (Oxford) with the following cyclic conditions: 95°C for 5 min, 30 cycles at 94°C for 30 sec, individual annealing temperature (Table 3) for 30 sec, 72°C for 30 sec, and a final extension of 72°C for 7 min. The amplified product of PCR assay was analysed by gel electrophoresis on a 1.5% agarose gel and stained with ethidium bromide for visualisation of gel under UV illumination.

Species-specific primers targeting the internal transcribed spacer-1 (ITS-1) region were used for initial PCR detection of *Eimeria* spp., as described by Haug et al. [20]. The species of *Eimeria* were confirmed by sequencing the representative PCR products obtained from PCR using species specific and universal primers (Table 3). PCR products were run on an

Table 3. PCR primers targeting the ITS-1 of the seven valid *Eimeria* species [52].

Species	Primer sequence 5' - 3'	Expected Amplicon Size (bp)	Annealing temperature (°C)
<i>Eimeria</i> spp. (universal primer)	5'-AAGTTGCGTAAATAGAGCCCT-3' 5'-AGACATCCATTGCTGAAAG-3'	400–750	56
<i>E. acervulina</i>	5'-GGGCTTGGATGATGTTTGCTG-3' 5'-GCAATGATGCTTGCACAGTCAGG-3'	145	57
<i>E. brunetti</i>	5'-CTGGGGCTGCAGCGACAGGG-3' 5'-ATCGATGGCCCCATCCCGCAT-3'	183	65
<i>E. maxima</i>	5'-TTGTGGGGCATATTGTTGTGA-3' 5'-CWCACCACTCACAATGAGGCAC-3'	162	55
<i>E. mitis</i>	5'-GTTTATTTCTGTCGTCTCGC-3' 5'-GTATGCAAGAGAGAATCGGGATTCC-3'	330	57
<i>E. necatrix</i>	5'-AGTATGGGCGTGAGCATGGAG-3' 5'-GATCAGTCTCATCATAATTCTCGCG-3'	160	53
<i>E. praecox</i>	5'-CATCGGAATGGCTTTTGAAGCG-3' 5'-GCATGCGCTAACAACCTCCCTT-3'	215	56
<i>E. tenella</i>	5'-AATTTAGTCCATCGCAACCCTTG-3' 5'-CGAGCGCTCTGCATACGACA-3'	278	55

<https://doi.org/10.1371/journal.pone.0327037.t003>

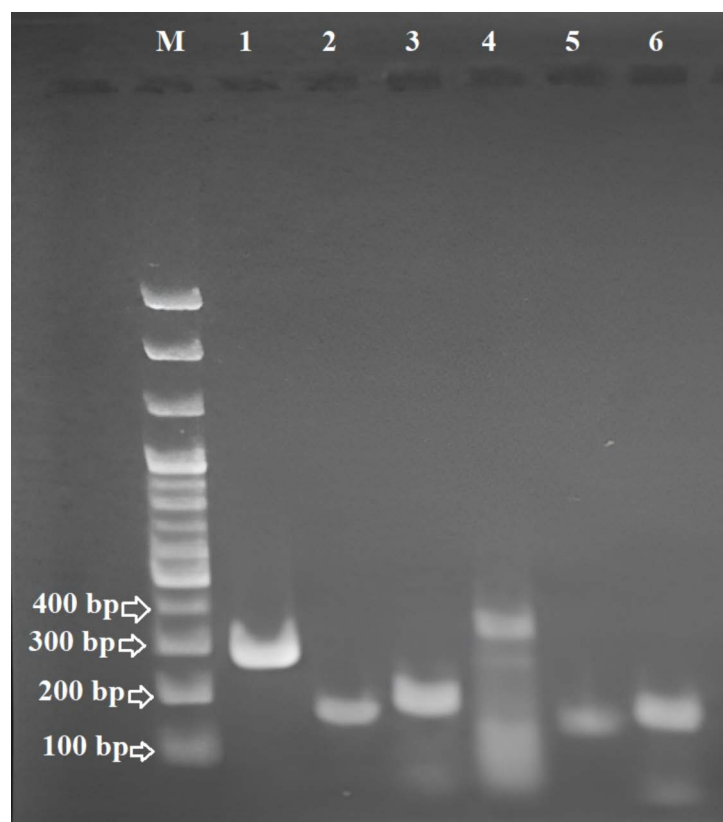


Fig 1. Agarose gel electrophoresis showing PCR amplification of representative *Eimeria* species from field samples. Lane M: 100 bp DNA ladder; Lane 1: *E. tenella* (278 bp) from Mymensingh (Sutiakhali); Lane 2: *E. acervulina* (146 bp) from Mymensingh (Vabokhali); Lane 3: *E. brunetti* (183 bp) from Rajshahi (Puba); Lane 4: *E. mitis* (330 bp) from Comilla (Sarail); Lane 5: *E. maxima* (162 bp) from Dhaka (Savar); Lane 6: *E. necatrix* (162 bp) from Rangpur (Gangachara).

<https://doi.org/10.1371/journal.pone.0327037.g001>

agarose gel, cut, purified, and then single-stranded products were generated using cycle sequencing PCR with forward or reverse primers. The purified products were run on a Sanger machine (ABI 3730 xl) using the dideoxy chain termination method at GeneCreate Biotech, China [21].

Representative sequences that were identified in this study were deposited in the GenBank (Accession No. LC855064–*Eimeria tenella*; LC855065 – *Eimeria necatrix*; LC855066 – *Eimeria brunetti*; LC855067– *Eimeria maxima*; LC855068– *Eimeria mitis* and LC855069– *Eimeria acervulina*)

Statistical analysis

The data were analyzed using statistical R software. Descriptive statistics were calculated to summarize the prevalence of coccidiosis across different regions and farming systems. The association between potential risk factors (age, sex, breed, rearing system and feed) and the presence of *Eimeria* oocysts was assessed using chi-square tests. A p-value of <0.05 was considered statistically significant.

Ethical considerations and permits

Fecal samples were collected non-invasively from chicken farm litter or from the ground surface in homestead free-ranging systems, without any handling or disturbance to the chickens. No animals were restrained, touched, or harmed during the sample collection process. Therefore, ethical approval was not required for this study. All samples were collected with prior verbal consent from farm owners or household heads. Since the study involved environmental sampling from private properties and did not include animal experimentation, no specific government permits were necessary. Field access was granted by the respective owners, and no protected areas or government-owned facilities were involved.

Results

Eimeria infection status in chickens

Of the 1500 faecal samples examined microscopically, *Eimeria* oocysts were detected in 87 (5.8%) samples out of 77 farms/flocks. The prevalence of *Eimeria* infection varied significantly across farms or flocks based on breed and management systems (Table 4). *Eimeria* oocysts were observed in 32 farms/flocks (41.56%) out of 77. Among exotic breeds, Broiler farms had a prevalence rate of 76.92% (10 out of 13 farms), followed by Sonali farms with a 66.67% prevalence (8 out of 12 farms). Fayoumi farms, however, reported no positive case. For native breeds, Aseel farms had the highest prevalence at 100% (5 out of 5 farms), followed by Deshi farms with 38.89% (7 out of 18 farms). Naked Neck and Hilly flocks demonstrated 0% prevalence (Table 2). In our study six species of *Eimeria* have been identified from various regions by PCR, including *E. tenella*, *E. acervulina*, *E. brunetti*, *E. mitis*, *E. necatrix*, and *E. maxima*.

Table 4. Risk factor analysis of *Eimeria* infection in different breeds of chicken.

Risk Factor	Chi Sq value	Category	Odds Ratio	95% CI	p-value
Breed	$\chi^2 = 20.76$, df=2, p<0.001	Exotic vs. Native	2.01	(1.29– 3.13)	0.002
		Mixed vs. Native	3.16	(0.93– 10.76)	0.066
Sex	$\chi^2 = 9.37$, df= 1, p= 0.002	Male vs. Female	2.07	(1.29– 3.32)	0.002
Age	$\chi^2 = 300.45$, df= 1, p<0.001	Young vs. Adult	200.76	(49.07–821.39)	<0.001
Rearing System	$\chi^2 = 66.25$, df= 1, p<0.001	Intensive with litter vs. Free range	8.13	(4.54– 14.56)	<0.001
Feed	$\chi^2 = 10.11$, df= 1, p=0.001	Commercial vs. Local	2.06	(1.32– 3.20)	0.001

Note: For the breed category, we used Native as the reference group for comparison. An odds ratio of 1 would indicate no association, while values further from 1 (in either direction) indicate stronger associations.

<https://doi.org/10.1371/journal.pone.0327037.t004>

Breed

The average Oocyst Per Gram (OPG) levels varied significantly across different breeds and management systems, reflecting the intensity of *Eimeria* infections. The prevalence of infection varied significantly across different breeds ($\chi^2=20.76$, $df=2$, $p<0.001$) with 7.96%, 4.13% and 12% infection of exotic, native and mixed population, respectively. The odds of infection in exotic breeds were significantly higher compared to native breeds (OR = 2.01, 95% CI: 1.29–3.13, $p=0.002$) (Table 4). Exotic breeds such as Broiler (13.98%) and Sonali (12.09%) showed significantly higher infection rates compared to native breeds like Deshi (3.40%) and Aseel (18.40%) ($p<0.05$). Fayoumi, Naked Neck and Hilly showed no cases of infection.

Among exotic breeds, Broilers had the highest average OPG range (33–1967) with 13.98% prevalence of infection, followed by Sonali (133–1066) with 12.09% prevalence, while Fayoumi showed no positive cases. For native breeds, Aseel had the highest average OPG (66–500) with 18.40% prevalence, whereas Deshi chickens exhibited lower OPG levels (33–133) with 3.40% prevalence. The mixed population also displayed varying OPG levels, with Broiler+Deshi combinations reaching 133 at the higher end.

Among *Eimeria* species *E. tenella* was detected in 64 samples (62.07%) and in all breeds with the highest occurrence in Aseel (23 cases) (Table 5). *E. acervulina* was the second most common species (25.28%), found in 22 samples from Deshi, Broiler and Sonali breeds. Other species, including *E. brunetti* (2.3%), *E. mitis* (3.45%), *E. necatrix* (3.45%), and *E. maxima* (3.45%), were less common and showed limited distribution across breeds. *E. tenella*, *E. acervulina*, and *E. necatrix* were detected in infected Deshi native chickens. *E. mitis* was detected in Broiler and Sonali birds while *E. brunetti* was identified exclusively in the Sonali breed. Notably, *E. necatrix* was detected in both Deshi native chickens and Broilers. Mixed infection was identified in two samples with *E. tenella*+ *E. mitis* infection only in Sonali and *E. acervulina*+ *E. maxima* in Broiler breeds.

Breed age was found to be the strongest risk factor associated with *Eimeria* infection ($\chi^2=300.45$, $df=1$, $p<0.001$). Young breeds had a substantially higher prevalence of 25.45%, whereas adult chickens had a prevalence of only 0.17% (OR = 200.76, 95% CI: 49.07–821.39, $p<0.001$). The wide confidence interval (49.07–821.39) suggests significant variability, likely due to the very low prevalence in adult chickens.

There was a significant association between sex and the prevalence of infection ($\chi^2=9.37$, $df=1$, $p=0.002$). Males had a higher prevalence (9.80%) compared to females (4.98%) with the odds of 2.07 (OR = 2.07, 95% CI: 1.29–3.32, $p=0.002$).

Rearing system

Samples were collected from various chicken types reared under different management systems in Bangladesh. Deshi chickens, typically raised as flocks in free-range systems. Naked Neck chickens were predominantly reared in free-range

Table 5. PCR detection of *Eimeria* species in microscopically positive samples from different breeds under different management systems.

<i>Eimeria</i> species	Breed; Positive samples (% infection)			
	Deshi (12)	Aseel (23)	Sonali (25)	broiler (27)
Total detection (87)				
<i>E. tenella</i> (54)	8	23	13	10
<i>E. acervulina</i> (22)	3	0	7	12
<i>E. brunetti</i> (2)	0	0	2	0
<i>E. mitis</i> (2)	0	0	1	2
<i>E. necatrix</i> (3)	1	0	0	2
<i>E. maxima</i> (3)	0	0	2	1
<i>E. tenella</i> + <i>E. mitis</i>	0	0	1	0
<i>E. acervulina</i> + <i>E. maxima</i>	0	0	0	1

<https://doi.org/10.1371/journal.pone.0327037.t005>

systems, except for two commercial flock kept in a semi-intensive system without litter. Aseel birds were exclusively raised in intensive systems with litter. Hilly chickens were primarily reared in free-range systems, with one flock managed semi-intensively without litter. All Sonali and Broiler chickens were kept in intensive systems with litter, whereas Fayoumi chickens were observed in intensive systems both with and without litter, as well as in semi-intensive systems with litter.

The rearing system was significantly associated with the prevalence of *Eimeria* infection ($\chi^2=66.25$, $df=1$, $p<0.001$). Chickens raised under intensive systems with litter had the a significantly higher prevalence (15.27%) compared to those in litter-free, semi-intensive, or free-range systems ($p<0.05$). The odds of coccidiosis were significantly higher in chickens raised in intensive systems with litter compared to those raised in a free-range system (OR = 8.13, 95% CI: 4.54–14.56, $p<0.001$). Notably, no oocysts were detected in samples collected from chickens reared in intensive systems without litter, semi-intensive systems with litter, or semi-intensive systems without litter. The highest prevalence of coccidiosis was observed in farms practicing a litter-based intensive system (68%), followed by intensive systems without litter (42.86%). Semi-intensive systems demonstrated prevalence rates of 30% with litter and 40% without litter.

When analyzed based on rearing systems, intensive farms with litter exhibited the highest prevalence at 68% (17 out of 25 farms), followed by Intensive without litter at 42.86% (3 out of 7) and free-range farms at 23.33% (7 out of 30 farms). Farms under semi-intensive systems with and without litter showed 30% and 40% prevalence, respectively.

Feed

The type of feed was found to have a significant impact on the prevalence of coccidiosis ($\chi^2=10.11$, $df=1$, $p=0.001$). Chickens fed commercial feed had a higher prevalence (7.88%) compared to those fed local feed (3.99%) with the odds of 2.06 (OR = 2.06, 95% CI: 1.32–3.20, $p=0.001$). The percentage of farms using coccidiostats varied across breeds and management systems. Among exotic breeds, 100% of Sonali and Broiler farms utilized coccidiostats, highlighting their intensive management practices and higher vulnerability to coccidiosis. Conversely, Fayoumi farms had a lower coccidiostat usage rate (60%). For native breeds, only Aseel farms consistently used coccidiostats (100%), while Deshi and Naked Neck farms had minimal usage rates of 5.56% and 6.67%, respectively (Table 2).

Discussion

Infection status of *Eimeria* in chicken

Rahman et al. [1] who reported 5.8% prevalence of coccidiosis in Gazipur and Badruzzaman et al. [22] reported 7.87% coccidiosis in Sylhet regions of Bangladesh which are in accordance with the current study. Our study identified six species of *Eimeria* (*E. tenella*, *E. acervulina*, *E. brunetti*, *E. mitis*, *E. necatrix*, and *E. maxima*) among the samples collected from different regions of Bangladesh. In a study by Alam et al. [17], five *Eimeria* species, including *E. tenella*, *E. necatrix*, *E. acervulina*, *E. brunetti*, and *E. mitis* were identified in broiler farms in Bangladesh using the ITS1 region specific marker. In contrast, Siddiki et al. [12] detected seven species, including *E. precox*. Neither this study nor Alam's study detected *E. precox*, which could be attributed to methodological differences, as Siddiki et al. [12] used conventional PCR targeting unique single-copy sequences derived from sequence-characterized amplified region (SCAR) markers. Research in Egypt isolated *E. tenella*, *E. acervulina*, *E. necatrix*, and *E. praecox* from native chickens [23] which in accordance with our study expect *E. praecox*. In India, next-generation sequencing revealed the presence of all seven recognized *Eimeria* species in both commercial and indigenous chickens, with *E. tenella* and *E. necatrix* dominating [24]. A study in Pakistan identified four *Eimeria* species in layer chickens, with *E. tenella* being the most prevalent (39.93%), and found higher infection rates in young chickens compared to adults [25].

Among *Eimeria* species *E. tenella* was detected in 62.07% of samples and in all breeds of this study. This finding is consistent with previous studies, such as Alzib et al. [26], which identified *E. tenella* as a highly pathogenic species responsible for severe cecal coccidiosis, particularly in intensively managed poultry systems. In Iran, a study found 64% of native chickens infected, with *E. tenella* being the most prevalent species [27]. Broilers showed the highest

diversity of *Eimeria* species, including *E. acervulina* (44.45%) and less common species like *E. mitis* and *E. necatrix* (7.41% each), indicating their susceptibility to multiple infections likely due to their intensive farming conditions [28]. Sonali chickens exhibited moderate prevalence rates, with infections of *E. brunetti* and *E. maxima* (8.33% each), underscoring their vulnerability under semi-intensive systems. Indigenous chicken varieties in Bangladesh, such as Common Deshi, Hilly, and Naked Neck, are known for their disease resistance and survivability [29]. In this study no or lower overall infection rates of native chicken, suggesting inherent resistance possibly linked to genetic and environmental factors [30]. None of the sample was positive for *E. praecox* which is in accordance with findings of Bhaskaran et al. [31] from India.

Breed

Studies have shown that exotic breeds, including Sonali and Broilers, tend to have higher prevalence rates compared to native breeds (Table 2). In Nigeria, exotic breeds exhibited a 42.4% prevalence rate, while in Bangladesh, coccidiosis was found to be the most common disease in Sonali chickens, with a prevalence of 21.27% [32,33]. Another study in Bangladesh reported coccidiosis as the most frequent disease in Sonali chickens, affecting 49.2% of cases [9]. These higher rates in exotic breeds may be attributed to poor management practices and biosecurity measures. Genetic predispositions and the management practices associated with exotic breeds often exacerbate their vulnerability. In contrast, native breeds like Deshi have shown lower susceptibility to various diseases, including coccidiosis [34]. In addition, the low prevalence is associated with extensive farming conditions, with gradual exposure to low levels of oocysts, and the development of immunity [35,36]. The relatively lower prevalence in Deshi chickens may indicate the potential for using indigenous breeds in coccidiosis management programs.

The study observed higher prevalence (9.80%) of male birds compared to females (4.98%). This result is consistent with the previous studies who reported a higher prevalence of poultry coccidiosis in male than female chickens [37]. Similarly, Wondimu et al. [38] reported higher prevalence of coccidiosis in male (43.6%) than female chicken (41.2%). However, most studies found no significant difference in prevalence between male and female chickens [39–41], although one study reported higher rates in females [42].

For coccidiosis, age is consistently identified as a major risk factor, with young chickens showing higher infection rates compared to adults [32,43,44]. Lower prevalence in older birds due to the immunity they develop from early exposure to the infection.

Rearing system

Chickens raised under intensive systems with litter had the highest prevalence (15.27%), while those reared in a free-range system had a much lower prevalence (2.17%). These results are consistent with findings from Negash et al. [43], Lawal et al. [32] and Adem et al. [45] where intensive systems were associated with poor litter management and overcrowding, providing favorable conditions for oocyst sporulation.

In contrast to commercial farms, free-range systems demonstrated a significantly lower prevalence of coccidiosis (7.14%). This is likely due to reduced bird density, natural feed sources, and minimal use of antibiotics or anticoccidials [46], which may help maintain healthier gut microbiota and stronger immunity in these chickens due to more natural rearing conditions [32]. In addition, the scavenging village chickens are also less likely to ingest pathogenic level of the coccidian oocysts during feeding [35,36].

Coccidiosis does not occur typically in all chickens in a flock simultaneously despite the presence of oocysts [47], as resistance and susceptibility vary among individuals [48]. Factors influencing the spread include population density, environmental conditions affecting oocyst development, and the development of immunity in previously infected birds [48]. However, the results underscore the higher risk of coccidiosis in farms with intensive management, exotic breeds, and mixed populations compared to native breeds and extensive systems.

Feed

Commercial poultry farms often rely on anticoccidial feed containing ionophores, non-ionophore chemicals, antibiotics, and growth promoters as part of their shuttle programs. Similarly, anticoccidial shuttle programs in Bangladesh are reliance on combinations of maduramycin, nicarbazin, salinomycin, and others. Among the coccidiostat used farms (38 farms), oocysts were detected in 32 farms (84.21%). Farms using commercial feed exhibited a higher prevalence of coccidiosis (55.10%) compared to free-range systems using local feed, which showed only 7.14% prevalence. Rony et al. [5] reported similar findings of higher coccidiosis prevalence in commercial systems of Bangladesh.

Coccidiostat usage was notably higher in intensive systems, particularly in those with litter, where 80% of farms included it in their practice which is in accordance with the finding of Kadykalo et al. [49]. Semi-intensive systems, both with and without litter, also had low coccidiostat usage rates (8% and 3%, respectively). This trend indicates that farms with intensive management and commercial feed are more dependent on coccidiostats, likely due to higher disease risks and production demands. Despite the use of coccidiostats, infections still occur with low OPG, because most of anticoccidial drugs just reduce the clinical symptoms but cannot inhibit the infections itself completely [50]. Therefore, the used anticoccidial drugs effectively reduced the OPGs and inhibited to develop the clinical symptoms in the present study.

Commercial feeds may contain subtherapeutic levels of anticoccidial drugs, which can lead to lower effectiveness against coccidiosis [51]. Countries with stringent biosecurity measures, improved management practices, and vaccination programs tend to report lower prevalence rates. The lack of such measures in Bangladesh, coupled with management practices, likely explains the higher prevalence in commercial systems.

Local feed used in free-range systems is associated with a very low prevalence in this study (Table 2). Local feed often contains natural ingredients that may improve gut health and immunity [52].

The overall lower rate of *Eimeria* infection reported in this study could reflect the current status of coccidiosis in Bangladesh, largely influenced by feeding and rearing practices in commercial poultry production. One critical factor contributing to this trend is the widespread use of feed supplemented with anticoccidial drugs. These medications are routinely administered as a preventive measure to control *Eimeria* infections, significantly reducing the prevalence and severity of coccidiosis in many broiler farms of Bangladesh.

Limitation of the study

This study has several limitations that should be acknowledged. The cross-sectional design captures prevalence at a single point in time, making it challenging to determine seasonal variations or long-term trends in *Eimeria* spp. infections. Additionally, reliance on fecal sampling, while useful for detecting oocysts, does not directly assess clinical severity or subclinical infections, potentially underestimating the true impact of coccidiosis. The molecular analysis, though effective for species identification, did not investigate genetic variations or emerging strains that could influence disease dynamics. Furthermore, data on management and feeding practices were collected through farmer surveys, which may be subject to recall bias or inaccuracies regarding feed formulations, anticoccidial usage, and disease prevention measures. Another important consideration is that native chickens reared in free-range systems consume a highly variable diet, which was not systematically analyzed for its potential influence on coccidiosis prevalence. Future studies should address these limitations by incorporating longitudinal sampling, histopathological analysis, genetic diversity assessments, and controlled dietary evaluations to provide a more comprehensive understanding of *Eimeria* infections in different poultry farming systems.

Conclusion

This study provides a comprehensive assessment of *Eimeria* infection prevalence in different chicken breeds and management systems across Bangladesh for the first time. The overall prevalence of *Eimeria* infection was 5.8%, with

significant variations across breeds, age groups, rearing systems, and feeding practices. Among exotic breeds, Broilers and Sonali chickens exhibited the highest infection rates, whereas Fayoumi chickens showed no infections. Among native breeds, Aseel chickens had the highest prevalence, while Naked Neck and Hilly chickens remained uninfected. The predominant *Eimeria* species identified were *E. tenella* and *E. acervulina*, with *E. brunetti*, *E. mitis*, *E. necatrix*, and *E. maxima* being less common.

The study highlights that younger birds are significantly more susceptible to infection, with males exhibiting a higher prevalence than females. Management systems played a crucial role, with intensive rearing on litter being the most significant risk factor for coccidiosis, while free-range systems demonstrated the lowest prevalence. Additionally, chickens fed commercial diets had a higher risk of infection compared to those consuming locally sourced feed.

These findings emphasize the need for targeted control measures, particularly in high-risk breeds and intensive farming systems. Improved biosecurity, optimized litter management, and strategic coccidiostat use could help mitigate *Eimeria* infections. Future research should focus on resistance mechanisms in naturally resistant breeds and explore alternative, sustainable approaches to coccidiosis prevention in poultry production.

Supporting information

S1_raw_images. Agarose gel electrophoresis showing PCR amplification of representative *Eimeria* species from field samples.

(PDF)

S2_raw_images. PCR Detection of *Eimeria* spp. from Field Samples.

(PDF)

Author contributions

Conceptualization: Md. Shahiduzzaman.

Data curation: Md. Afazur Rahman, Kazi Farah Tasfia, Makoto Matsubayashi.

Formal analysis: Md. Afazur Rahman, A R M Beni Amin, Kazi Farah Tasfia, Makoto Matsubayashi.

Funding acquisition: Md. Shahiduzzaman.

Investigation: Md. Shahiduzzaman, Kazi Farah Tasfia.

Methodology: Md. Shahiduzzaman, Md. Afazur Rahman, A R M Beni Amin, Kazi Farah Tasfia.

Project administration: Md. Shahiduzzaman.

Supervision: Md. Shahiduzzaman.

Validation: A R M Beni Amin, Kazi Farah Tasfia.

Visualization: Makoto Matsubayashi.

Writing – original draft: Md. Shahiduzzaman.

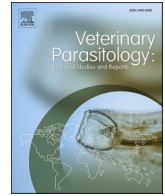
Writing – review & editing: Md. Shahiduzzaman, Makoto Matsubayashi.

References

1. Rahman MA, Rahman MM, Moonmoon M, Alam KJ, Islam MZ. Prevalence of common diseases of broiler and layer at Gazipur district in Bangladesh. Asian J Med Biol Res. 2017;3(2):290–3.
2. Bangladesh Poultry Industries Coordination Committee BPICC. Annual report, 2021. 2021.
3. Farooq M, Durrani FR, Waheedullah W, Sajjad A, Asghar A. Prevalence of coccidiosis in broilers in the subtropical environment. 1999. <http://www.priory.com/vet/broilers.htm>

4. Chowdhury SD. Family poultry production in Bangladesh: is it meaningful or an aimless journey?. *World's Poultry Science Journal*. 2013;69(3):649–65. <https://doi.org/10.1017/s0043933913000652>
5. Rony SA, Islam M, Alam M. Small-scale farmers' perception and practice on coccidiosis management in broiler farms at Gazipur, Bangladesh. *Ann Parasitol*. 2021;67(1):315.
6. Rashid MH, Xue C, Islam MR, Cao Y. Risk factors associated with infectious bursal disease in commercial chickens in Bangladesh. *Prev Vet Med*. 2013;111(1–2):92–100.
7. Dolberg F. Poultry sector country review: Bangladesh. 2008. <http://www.fao.org/3/aak069e.pdf>
8. Parvin R, Nooruzzaman M, Kabiraj CK, Begum JA, Chowdhury EH, Islam MR, et al. Controlling avian influenza virus in Bangladesh: challenges and recommendations. *Viruses*. 2020;12(7):751. <https://doi.org/10.3390/v12070751> PMID: 32664683
9. Islam M, Singha S, Belgrad JP, Hasib EMY, Sayeed MA, Haque ME, et al. Common chicken diseases in Kishoreganj, Bangladesh: estimation through the veterinary hospital-based passive surveillance system. *Adv Anim Vet Sci*. 2021;9(11):1951–8.
10. Masud AA, Rousham EK, Islam MA, Alam M-U, Rahman M, Mamun AA, et al. Drivers of Antibiotic Use in Poultry Production in Bangladesh: Dependencies and Dynamics of a Patron-Client Relationship. *Front Vet Sci*. 2020;7:78. <https://doi.org/10.3389/fvets.2020.00078> PMID: 32185184
11. Tasmim ST, Hasan MM, Talukder S, Mandal AK, Parvin MS, Ali MY, et al. Sociodemographic determinants of use and misuse of antibiotics in commercial poultry farms in Bangladesh. *IJD Reg*. 2023;7:146–58. <https://doi.org/10.1016/j.ijregi.2023.01.001> PMID: 37082426
12. Siddiki AZ, Mina S, Anayet Hasan Md, Touaha Akbar M, Alam R, Ashrafu Islam Md, et al. Molecular characterization of *Eimeria* spp. from chicken by Polymerase Chain Reaction based on species-specific SCAR markers. *IOSR JAVS*. 2014;7(1):13–7. <https://doi.org/10.9790/2380-07111317>
13. Gadelhaq SM, Arafa WM, Aboelhadid SM. Molecular characterization of *Eimeria* species naturally infecting egyptian baldi chickens. *Iran J Parasitol*. 2015;10(1):87–95. PMID: 25904950
14. Khaier MA, Daffala HA, Abukashawa S. Isolation, identification and molecular characterization of *Eimeria* spp. infecting chickens in Khartoum State, Sudan using ITS1 gene. *J Bioinform Mol Biol*. 2020;5(1):1–7.
15. Belal SMSH. prevalence of coccidiosis in sonali birds in sirajgonj district of Bangladesh. *Bangl J Vet Med*. 2018;15(2):107–11. <https://doi.org/10.3329/bjvm.v15i2.35519>
16. Talukdar ML, Zuhra FT, Islam KME, Ahmed MS. Prevalence of infectious diseases in Sonali chickens at Bogra Sadar Upazila, Bogra, Bangladesh. *J Adv Vet Anim Res*. 2017;4(1):39–44.
17. Alam MZ, Dey AR, Parvin S, Akter S, Rony SA. ITS1-PCR based identification of chicken *Eimeria* species in poultry litter from Mymensingh district, Bangladesh. *J Adv Vet Anim Res*. 2021;8(3):489–93. <https://doi.org/10.5455/javar.2021.h538> PMID: 34722748
18. Hodgson JN. Coccidiosis: oocyst counting technique for coccidiostat evaluation. *Exp Parasitol*. 1970;28(1):99–102. [https://doi.org/10.1016/0014-4894\(70\)90073-1](https://doi.org/10.1016/0014-4894(70)90073-1) PMID: 5459879
19. Conway DP, Mckenzie ME. Poultry Coccidiosis: Diagnostic and Testing Procedures. 3rd ed. Ames, Iowa: Blackwell Publishing. 2007.
20. Haug A, Thebo P, Mattsson JG. A simplified protocol for molecular identification of *Eimeria* species in field samples. *Vet Parasitol*. 2007;146(1–2):35–45. <https://doi.org/10.1016/j.vetpar.2006.12.015> PMID: 17386979
21. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA*. 1977;74:5463–7.
22. Badruzzaman ATM, Noor M, Al Mamun M, Husna A, Islam KM, Rahman MM. Prevalence of diseases in commercial chickens at Sylhet division of Bangladesh. *Int Clin Pathol J*. 2015;1(5):23.
23. Amer MM, Awaad MHH, El-Khateeb RM, Abu-Elezz NMTN, Said AS, Ghetas MM, et al. Isolation and identification of *Eimeria* from field coccidiosis in chickens. *J Am Sci*. 2010;6(10):1221–6.
24. Bachaya HA, Raza MA, Khan MN, Iqbal Z, Abbas RZ, Murtaza S, et al. Predominance and detection of different *Eimeria* species causing coccidiosis in layer chickens. *J Anim Plant Sci*. 2012;22(3):597–600.
25. Hinsu AT, Thakkar JR, Koringa PG, Vrba V, Jakhesara SJ, Psifidi A, et al. Illumina Next Generation Sequencing for the Analysis of *Eimeria* Populations in Commercial Broilers and Indigenous Chickens. *Front Vet Sci*. 2018;30(5):176.
26. Alzib AA, Abdelnabi GH. *Eimeria* spp. infection in some broiler farms in Khartoum State, Sudan. *SOJ Vet Sci*. 2017;3(4):1–3.
27. Mohammad MH, Ahad O, Mohammad N, Fariborz A, Omid N. Prevalence of *Eimeria* species in scavenging native chickens of Shiraz, Iran. *Afr J Microbiol Res*. 2011;5(20):3296–9. <https://doi.org/10.5897/ajmr11.477>
28. Shirley MW, Smith AL, Tomley FM. The biology of avian *Eimeria* with an emphasis on their control by vaccination. *Adv Parasitol*. 2005;60:285–330. [https://doi.org/10.1016/S0065-308X\(05\)60005-X](https://doi.org/10.1016/S0065-308X(05)60005-X) PMID: 16230106
29. Rabbani MAG, Vallejo-Trujillo A, Wu Z, Miedzinska K, Faruque S, Watson KA, et al. Whole genome sequencing of three native chicken varieties (Common Deshi, Hilly and Naked Neck) of Bangladesh. *Sci Data*. 2024;11(1):1432. <https://doi.org/10.1038/s41597-024-04291-z> PMID: 39719437
30. Pinard-van der Laan M-H. Immune modulation: the genetic approach. *Vet Immunol Immunopathol*. 2002;87(3–4):199–205. [https://doi.org/10.1016/s0165-2427\(02\)00075-2](https://doi.org/10.1016/s0165-2427(02)00075-2) PMID: 12072235
31. Bhaskaran MS, Venkatesan L, Aadimoolam R, Tirunelveli Jayagopal H, Sriraman R. Sequence diversity of internal transcribed spacer-1 (ITS-1) region of *Eimeria* infecting chicken and its relevance in species identification from Indian field samples. *Parasitol Res*. 2010;106(2):513–21. <https://doi.org/10.1007/s00436-009-1696-2> PMID: 20012096

32. Lawal JR, Jajere SM, Ibrahim UI, Geidam YA, Gulani IA, Musa G, et al. Prevalence of coccidiosis among village and exotic breed of chickens in Maiduguri, Nigeria. *Vet World*. 2016;9(6):653–9. <https://doi.org/10.14202/vetworld.2016.653-659> PMID: [27397991](#)
33. Tipu JH, Al Mamun M, Noor M, Ahsan MI, Bhuiyan MJU. Prevalence and pathological affections of infectious diseases in Sonali chickens in the Kishoreganj district of Bangladesh. *Adv Anim Vet Sci*. 2021;9(9):1317–23.
34. Islam MS, Dutta RK. Egg quality traits of indigenous, exotic and crossbred chickens (*Gallus domesticus* L.) in Rajshahi, Bangladesh. *J Life Earth Sci*. 1970;5:63–7. <https://doi.org/10.3329/jles.v5i0.7352>
35. Gari G, Tilahun G, Dorchies PH. Study on poultry coccidiosis in Tiyo District, Arsi zone, Ethiopia. *Int J Poult Sci*. 2008;7(3):251–6.
36. Oljira D, Melaku A, Bogale B. Prevalence and risk factors of coccidiosis in poultry farms in and around Ambo Town, Western Ethiopia. *Am Euras J Sci Res*. 2012;7(4):146–9.
37. Hadas G, Merhatu G, Abebe T. Prevalence of poultry coccidiosis in Gondar town, Ethiopia. *Eurasian J Educ Res*. 2013;9(5):129–35.
38. Wondimu A, Mesfin E, Bayu Y. Prevalence of Poultry Coccidiosis and Associated Risk Factors in Intensive Farming System of Gondar Town, Ethiopia. *Vet Med Int*. 2019;2019:5748690. <https://doi.org/10.1155/2019/5748690> PMID: [32089814](#)
39. Akalu A. Addis-Ababa-Arada sub-city administration trade office urban-agricultural core process case team of animal health, Ethiopia. *Br Poult Sci*. 2017;6(3):80–6.
40. Addis K, Endale T. Prevalence of poultry coccidiosis in and around Yabello, Southern Ethiopia. *World J Agric Res*. 2016;12(5):342–5.
41. Lawal JR, Jajere SM, Ibrahim UI, Geidam YA, Gulani IA, Musa G, et al. Prevalence of coccidiosis among village and exotic breed of chickens in Maiduguri, Nigeria. *Vet World*. 2016;9(6):653–9. <https://doi.org/10.14202/vetworld.2016.653-659> PMID: [27397991](#)
42. Usman AM, Malann YD, Babeker EA. Prevalence of Coccidiosis among local and exotic breeds of reared chickens in Azare Metropolis, Bauchi State Nigeria. *dujopas*. 2022;8(3b):109–14. <https://doi.org/10.4314/dujopas.v8i3b.11>
43. Negash A, Mohamed A, Wondimu K. Study on prevalence and risk factors associated with poultry coccidiosis in and around Hawassa Town, South Ethiopia. *Br J Poult Sci*. 2015;4(2):34–43.
44. Ali H, Naqvi F, Tariq N. Prevalence of coccidiosis and its association with risk factors in poultry of Quetta, Pakistan. *Asian J Appl Sci*. 2014;2(4):554.
45. Adem DM, Ame MM. Prevalence of poultry coccidiosis and its associated risk factors in and around Haramaya District, Ethiopia. *Vet Med Open J*. 2023;8(1):9–17. <https://doi.org/10.17140/vmoj-8-172>
46. Ferdushy T, Hasan MT, Golam Kadir AKM. Cross sectional epidemiological investigation on the prevalence of gastrointestinal helminths in free range chickens in Narsingdi district, Bangladesh. *J Parasit Dis*. 2016;40(3):818–22. <https://doi.org/10.1007/s12639-014-0585-5> PMID: [27605790](#)
47. Chapman HD, Barta JR, Hafeez MA, Matsler P, Rathinam T, Raccoursier M. The epizootiology of *Eimeria* infections in commercial broiler chickens where anticoccidial drug programs were employed in six successive flocks to control coccidiosis. *Poult Sci*. 2016;95(8):1774–8.
48. Horton-Smith C. Some factors influencing the origin and course of epidemics of coccidiosis in poultry. *Ann New York Acad Sci*. 1949;52(4):449–57. <https://doi.org/10.1111/j.1749-6632.1949.tb53928.x>
49. Kadykalo S, Roberts T, Thompson M, Wilson J, Lang M, Espeisse O. The value of anticoccidials for sustainable global poultry production. *Int J Antimicrob Agents*. 2018;51(3):304–10. <https://doi.org/10.1016/j.ijantimicag.2017.09.004> PMID: [28935212](#)
50. Kant V, Singh P, Verma PK, Bais I, Parmar MS, Gopal A, et al. Anticoccidial drugs used in poultry: an overview. *Sci Int*. 2013;1(4):261–5.
51. Martins RR, Silva LJG, Pereira AMPT, Esteves A, Duarte SC, Pena A. Coccidiostats and poultry: a comprehensive review and current legislation. *Foods*. 2022;11(18):2738. <https://doi.org/10.3390/foods11182738> PMID: [36140870](#)
52. Colombino E, Biasato I, Ferrocino I, Bellezza Oddon S, Caimi C, Gariglio M, et al. Effect of insect live larvae as environmental enrichment on poultry gut health: gut mucin composition, microbiota and local immune response evaluation. *Animals (Basel)*. 2021;11(10):2819. <https://doi.org/10.3390/ani11102819> PMID: [34679839](#)



Original Article

Comparative susceptibility of native chicken breeds of Bangladesh to experimental *Eimeria tenella* infection

Dipesh Aryal, Kazi Farah Tasfia, Al Nur Tarak, Asmita Bhujel, Md. Shahiduzzaman *

Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ARTICLE INFO

Keywords:

Eimeria
Coccidiosis
Chickens
Pathology
Resistance
Indigenous
qPCR

ABSTRACT

Eimeria tenella is the major protozoan parasite that causes coccidiosis in chickens, and its clinical and pathological response shows significant variations among breeds. This disease has a major economic impact on growers and the poultry industry worldwide. Very little information exists concerning the challenge infections of native chicken breeds of Bangladesh. The present study was undertaken to investigate and compare the pathology and clinical manifestations of experimental *E. tenella* infection among native (indigenous) chicken breeds of Bangladesh (Hilly, Naked Neck, and Deshi), in comparison to the susceptible exotic Sonali breed. In order to achieve this, a controlled experimental infection was carried out, and weight changes, Feed Conversion ratio (FCR), OPG counts, lesion severity, qPCR quantification of oocysts output, and dropping score were measured at 6, 10, and 14-Days post-infection (DPI). Distinct breed-specific differences in susceptibility were elicited. Sonali chickens exhibited the highest oocyst counts, reaching up to 103,200 at 14 DPI, along with significant weight loss and severe cecal pathology marked by diffuse hemorrhagic lesions. On the other hand, the indigenous breeds had low OPG counts as low as 100 in Hilly chickens, insignificant changes in weights, and mild pathological features characterized by reduced inflammation and hemorrhagic enteritis. This study highlights significant breed-specific differences in susceptibility to *E. tenella*, offering valuable insights into potential resistance or tolerance mechanisms. These findings support the use of native breeds in selective breeding programs aimed at improving disease resistance and underscore the importance of targeted control strategies for effective coccidiosis prevention and management.

1. Introduction

Coccidiosis is one of the most economically significant and widespread gastrointestinal diseases affecting poultry worldwide (Dalloul and Lillehoj, 2006). It is caused by protozoan parasites of the genus *Eimeria*, which are obligate intracellular pathogens characterized by a complex and host-specific life cycle. The disease leads to considerable morbidity and mortality in chickens, thereby compromising animal welfare and resulting in substantial economic losses due to decreased productivity, poor feed conversion, and the cost of treatment and prophylaxis (Blake et al., 2020). Among the seven *Eimeria* species known to infect chickens, *Eimeria tenella* is considered one of the most pathogenic, primarily targeting the ceca and causing severe hemorrhagic lesions (Attia et al., 2023). Clinical manifestations include intestinal bleeding, emaciation, loss of pigmentation, bloody cecal cores, and high mortality, often accompanied by clusters of schizonts and oocysts in the cecal mucosa (Habibi et al., 2016).

In Bangladesh, commercial poultry farming has expanded rapidly since the 1980s and now contributes over 50 % to the country's total meat production (Raha, 2007). Despite this growth, there remains a critical research gap regarding breed-specific susceptibility to *E. tenella*, particularly among native chicken breeds. Most published studies focus on exotic or commercial hybrids, leaving the resilience and pathological responses of indigenous breeds underexplored. Understanding how native breeds respond to *Eimeria* infection is essential for developing targeted control strategies, guiding resistance best breed selection, and improving disease management in low input farming systems.

Native chicken breeds, such as the Hilly, Naked Neck, and Deshi (non-descriptive local), are presumed to possess greater disease tolerance due to their genetic diversity and adaptation to local environments (Rabbani et al., 2024). The Hilly breed, native to the Chittagong Hill Tracts, is known for its ruggedness, disease resistance and foraging ability (Monira and Hussain, 2020; Uddin et al., 2011). The Naked Neck breed offers superior heat tolerance and growth efficiency, traits well

* Corresponding author.

E-mail address: szaman@bau.edu.bd (Md. Shahiduzzaman).<https://doi.org/10.1016/j.vprsr.2025.101353>

Received 16 June 2025; Received in revised form 18 September 2025; Accepted 26 September 2025

Available online 28 September 2025

2405-9390/© 2025 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

suited to Bangladesh's hot, humid climate (Barua et al., 1998; Islam and Nishibori, 2009). Deshi chickens, widely reared in rural households, are valued for their meat quality, broodiness, and ability to thrive on household scraps (Yeasmin and Howlider, 1998). These characteristics make native breeds a practical and sustainable choice for smallholder and medium scale farmers, offering resilience against disease, lower production costs, and vital contributions to rural livelihoods.

This study was designed to address the lack of comparative data on native Bangladeshi chicken breeds in relation to *E. tenella* infection. Specifically, it aims to investigate and compare the pathological and clinical manifestations of experimental *E. tenella* infection among three native breeds (Hilly, Naked Neck, and Deshi) and one exotic breed (Sonali), which is widely used in commercial production. The objective is to assess breed specific susceptibility using a comprehensive set of parameters: body weight changes, feed conversion ratio (FCR), oocyst per gram (OPG) counts, lesion scores, quantitative PCR (qPCR) for parasite load, and dropping score. We hypothesize that native chicken breeds will exhibit greater resistance to *E. tenella* infection compared to the exotic Sonali breed under controlled experimental conditions, thereby providing insights for sustainable breed selection and coccidiosis management in Bangladesh.

2. Materials and methods

2.1. Birds and management

The study was carried out at the poultry shed of the Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University. Four chicken breeds were used: three native breeds (Hilly, Naked Neck, and Deshi-local non descriptive) and one exotic crossbreed (Sonali), encompassing both exotic and native varieties commonly raised by farmers across various regions of Bangladesh. A total of 108-day-old chicks (27 per breed) were included. Sonali (commonly reared in Bangladesh due to their excellent meat quality) and desi chickens were procured from a reputable local hatchery at the age of 7 days while Hilly and Naked Neck chicks were obtained from the Bangladesh Livestock Research Institute (BLRI) at the age of 7 days. After 4 days of acclimation, birds were housed in a cage system under controlled management. Each experimental unit consisted of three birds per pen, with nine replicate pens per breed. The cages measured 90 cm in length, 70 cm in width, and 50 cm in height, providing adequate space for movement, feeding, and behavioral observation. Pens were thoroughly cleaned and disinfected prior to the trial. Dry wood shavings were used as a litter and replaced every two days to maintain hygiene and minimize moisture. All birds were provided with ad libitum access to feed and water throughout the experiment. The birds were fed a standard commercial Sonali starter diet, the composition of which was maize (62.8 %), soybean meal-45.8 % (29.759 %), full fat soya-37 % (2 %), dried distillers grains with solubles (DDGS) (1 %), rice bran oil (0.8 %), salt (0.3 %), limestone powder (1.3 %), and monocalcium phosphate (0.7 %). The formulated diet contained 20 % crude protein (CP) and 2400 kcal/kg metabolizable energy (ME), supplemented with vitamins and minerals, and was free from anticoccidial drugs. Daily feed offered and refusals were weighed to calculate feed intake per replicate pen. Birds were vaccinated according to standard protocols against Newcastle disease and infectious bursal disease.

2.2. *Eimeria* isolate and confirmation

Fecal samples suspected of *Eimeria tenella* infection were collected from various farms. Oocysts were isolated by centrifugal flotation and sporulated in 2.5 % potassium dichromate at room temperature (25 °C). Sporulated oocyst were stored at 4 °C and used within one month. Infectivity was confirmed by orally inoculating three-week-old *Eimeria* free six broiler chicks with 7×10^4 sporulated oocysts per bird. Four days post-infection, the birds exhibited typical clinical signs of

coccidiosis (bloody diarrhea, anorexia, somnolence) with 20 % mortality. Necropsy confirmed cecal hemorrhages, clotted blood, cecal core formation, and mucosal thickening consistent with *E. tenella* (Sagolsem et al., 2021). After 10 days post-infection, sufficient oocysts were recovered from the feces. DNA was extracted from infected cecal tissue using a commercial kit, and Polymerase Chain Reaction (PCR) was performed to amplify the *E. tenella* gene. The resulting PCR product was submitted for gene sequencing to confirm *E. tenella*.

2.3. Experimental infection

All chickens were screened for the presence of *Eimeria* oocysts two days prior to the experimental infection and were confirmed to be free of natural infection. A total of 108 chickens were used (27 per breed). In each group, six birds per replicate pen were orally infected with 7×10^4 sporulated *E. tenella* oocysts per bird using a clean dropper. The remaining three birds per group served as uninfected controls. Fresh droppers were used for each inoculation to prevent cross-contamination. Birds were kept in a cage system, housed in groups within designated pens, with three birds per pen to maintain replicates. Birds in each experimental group were housed separately in designated pens (Table 1).

2.4. Monitoring and sample collection

Clinical signs (lethargy, bloody feces, reduced feed intake, weight loss, mortality) were recorded daily. Fecal samples were collected from each pen starting on Day 4 post-infection and oocyst counts (OPG) were determined using the McMaster technique after flotation in saturated salt solution (Henriksen and Aagaard, 1976).

Weight gain was calculated as the difference between final and initial weights at each DPI. The daily feed given to each group of birds was weighed and the amount left in the feeders was also weighed the following day to determine the daily feed intake and Feed Conversion Ratio (FCR) was calculated as feed intake divided by weight gain (Holdsworth et al., 2004).

At 6, 10, and 14 days post-infection (DPI), birds were euthanized using manual cervical dislocation, performed in accordance with institutional animal care and use guidelines of Bangladesh Agricultural University. This method ensured rapid loss of consciousness and minimized pain and distress during sample collection for lesion scoring, histopathology, and quantification was performed by qPCR analysis. Lesions were scored from 0 to 4 using the method described by (Conway and McKenzie, 2007). Dropping scores were graded on a scale of 0–4 considering the presence of mucus and/or blood as described by (Johnson and Reid, 1970).

2.5. Histopathology

Cecal tissues were collected for histopathological analysis. Samples were fixed in 10 % neutral buffered formalin, dehydrated in ethanol, cleared in xylene, embedded in paraffin, and sectioned at 5 µm. Sections were stained with hematoxylin and eosin (Lillie, 1965). The sections were examined under 400 × magnification for histopathological changes and parasite stages (schizonts, gametocytes, and oocysts).

2.6. Quantification of infection by qPCR

DNA was extracted from combined cecal and fecal contents using the PureLink® Genomic DNA Extraction Kit (Thermo Fisher, USA). Quantitative real-time PCR was performed with species-specific primers for *E. tenella* after experimental infection (Blake et al., 2008): TEN-F: TCGTCTTTGGCTGGCTATTG; TEN-R: CAGAGATCGCCGTCACAGT. Quantitative real-time PCR was performed using Mic qPCR (Biomolecular systems, Australia). The total volume of 20 µl contained 10 µl of SYBR Green Master Mix (Takara, Japan), 0.8 µl of species-specific

Table 1
Effect of *Eimeria tenella* infection on body weight gain and feed conversion ratio (FCR) of different indigenous chicken breeds at 6, 10, and 14 days post-infection (DPI) (n = 108).

Days Post Infection (DPI)		6 DPI		10 DPI		14 DPI	
Breed	Infection status	Weight Gain (g)	FCR	Weight Gain (g)	FCR	Weight Gain (g)	FCR
Sonali	Control	68 ± 3.77	2.85 ± 0.16	127 ± 0.94	2.88 ± 0.02	181 ± 2.83	3.05 ± 0.05
	Infection	59 ± 2.07	3.28 ± 0.11	98 ± 2.26 ^{ab}	3.75 ± 0.09 ^a	122 ± 1.18 ^{bc}	4.53 ± 0.04 ^a
Hilly	Control	62 ± 4.71	3.27 ± 0.25	101 ± 2.36	3.71 ± 0.09	135 ± 7.07	4.18 ± 0.22
	Infection	60 ± 5.27	3.49 ± 0.33	98 ± 3.31 ^b	3.84 ± 0.13 ^{ab}	132 ± 4.29 ^{ab}	4.27 ± 0.14 ^{ab}
Naked Neck	Control	68 ± 5.66	2.99 ± 0.25	114 ± 5.66	3.32 ± 0.17	160 ± 18.86	3.67 ± 0.45
	Infection	63 ± 3.06	3.21 ± 0.16	93 ± 5.65 ^b	4.14 ± 0.26 ^{ab}	146 ± 6.95 ^c	3.9 ± 0.19 ^a
Deshi	Control	57 ± 4.71	3.57 ± 0.3	96 ± 5.66	3.96 ± 0.24	126 ± 6.6	4.5 ± 0.24
	Infection	56 ± 4.05	3.67 ± 0.28	80 ± 5.27 ^a	4.83 ± 0.33 ^b	123 ± 3.61 ^a	4.59 ± 0.13 ^b

Values are presented as mean ± standard error (SE). Different superscript letters (a, b, c) within the same column at each DPI indicate statistically significant differences between breeds (*p* < 0.05).

primers, 0.4 µl of ROX passive reference dye, 6 µl of nuclease-free water, and 2 µl of template DNA. Cycling conditions for amplification were as follows: 95 °C initial denaturation for 1 min, followed by 40 cycles of 95 °C for 15 s and 55 °C for 60 s. Standard curves were generated using serial dilutions of *E. tenella* genomic DNA, and parasite load (per gram) was calculated from Cq values using Mic qPCR software. Biomolecular grade water was used as no template negative control.

2.7. Data analysis

All statistical analyses were performed using IBM SPSS Statistics (version 27; IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard error. Normality of the data was assessed using Shapiro-Wilk test. Parasitological and pathological parameters, including oocyst counts, RT-PCR gene copies, lesion scores, and dropping scores, which deviated from normality, were compared among breeds using the Kruskal–Wallis test. Growth performance parameters specifically body weight gain (WG) and feed conversion ratio (FCR) at 6, 10, and 14 days post-infection, were analyzed using a general linear model (GLM) with breed and infection status included as fixed factors and their interaction tested in the model. Homogeneity of variances was verified using Levene's test. For post-hoc comparison, Duncan's multiple range test was applied. All the statistical significance was considered at *p* < 0.05.

3. Results

The impact of *Eimeria tenella* infection was evaluated in four indigenous chicken breeds-Sonali, Hilly, Naked Neck, and Deshi at 6, 10, and 14 DPI, focusing on body weight gain, feed conversion ratio (FCR), oocyst shedding, qPCR parasite burden, lesion development, and fecal appearance (Tables 1 and 2).

3.1. Clinical signs, weight gain, and feed efficiency

Sonali chickens exhibited the highest susceptibility to *E. tenella*. At 6 DPI, they were the only breed with detectable oocyst shedding (267 ± 14.43 OPG), a qPCR gene copy number of 6.15 ± 0 log₁₀, and mild lesion (score 1) with loose droppings (score 2). Weight gain was slightly reduced (59 ± 2.07 g) compared to controls (68 ± 3.77 g), with a modest increase in FCR (3.28 ± 0.11 vs. 2.85 ± 0.16). By 10 DPI, oocyst output rose sharply (4361 ± 163.05 OPG), with elevated qPCR parasite burden (6.85 ± 0 log₁₀), and lesion scores (score 3) and fecal scores (score 3). The infection significantly affected weight gain (98 ± 2.26 g vs. 127 ± 0.94 g in controls) and FCR (3.75 ± 0.09 vs. 2.88 ± 0.02). At 14 DPI, oocyst shedding peaked (103,200 ± 3333.54 OPG), accompanied by severe intestinal lesions (score 4), watery droppings (score 4), and the highest parasite gene copy number (8.11 ± 0 log₁₀). Sonali chickens recorded the lowest weight gain (122 ± 1.18 g) and poorest FCR (4.53 ± 0.04), underscoring their high sensitivity to infection.

Table 2
Comparative parasitological (oocyst per gram of feces, OPG), molecular (qPCR quantification), and clinical (lesion score and dropping score) parameters of different indigenous chicken breeds following *Eimeria* infection at 6, 10, and 14 days post-infection (DPI) (n = 108).

DPI	Parameters	Sonali	Hilly	Naked Neck	Deshi
6	Avg. OPG	267 ± 14.43 ^b	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
	qPCR (Log ₁₀ copies/g)	6.15 ± 0 ^b	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
	Lesion Score	1 ± 0 ^b	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
	Dropping Score	2 ± 0 ^b	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
	Avg. OPG	4361 ± 163.05 ^c	100 ± 8.25 ^a	2980 ± 200.03 ^b	1700 ± 175.71 ^b
10	qPCR (Log ₁₀ copies/g)	6.85 ± 0 ^b	6.11 ± 0.01 ^a	6.37 ± 0.02 ^{ab}	6.32 ± 0 ^{ab}
	Lesion Score	3 ± 0 ^c	0 ± 0 ^a	1 ± 0 ^b	1 ± 0 ^b
	Dropping Score	3 ± 0 ^c	0 ± 0 ^a	1 ± 0 ^b	0 ± 0 ^b
	Avg. OPG	103,200 ± 3333.54 ^c	540 ± 31.84 ^a	68 ± 11.01 ^a	1200 ± 59.98 ^b
	qPCR (Log ₁₀ copies/g)	8.11 ± 0 ^c	6.19 ± 0 ^a	5.66 ± 0 ^a	6.29 ± 0.01 ^b
14	Lesion Score	4 ± 0 ^c	1 ± 0 ^b	0 ± 0 ^a	1 ± 0 ^b
	Dropping Score	4 ± 0 ^c	0 ± 0 ^b	0 ± 0 ^a	0 ± 0 ^b

Values are presented as mean ± standard error (SE). Different superscript letters (a, b, c) within the same row indicate statistically significant differences between breeds at the same DPI (*p* < 0.05).

Statistically significant differences in FCR were found at both 10 DPI and 14 DPI.

Hilly chickens demonstrated strong resistance to *E. tenella*. They remained asymptomatic and shed no oocysts at 6 DPI. At 10 DPI, oocyst output was low (100 ± 8.25 OPG), with no visible clinical signs. At 14 DPI, oocyst shedding increased modestly (540 ± 31.84 OPG), with mild lesions (score 1), but weight gain remained relatively unaffected (132 ± 4.29 g), and FCR stayed stable. No statistically significant differences in FCR were detected at any time point.

Naked Neck chickens showed early susceptibility but quickly developed resistance. At 6 DPI, there were no detectable oocysts or lesions. By 10 DPI, oocyst output increased (2980 ± 200.03 OPG), with mild lesion (score 1) and fecal scores (score 1), and exhibited significantly reduced body weight gain (93 ± 5.65 g) compared to their controls (114 ± 5.66 g; *p* < 0.05), accompanied by a tendency toward higher FCR (4.14 ± 0.26 vs. 3.32 ± 0.17). However, at 14 DPI, oocyst shedding dropped drastically (68 ± 11.01 OPG), with mild visible lesions and normal droppings. This breed showed higher weight gain among infected birds at 14 DPI (146 ± 6.95 g) than Hilly and Deshi and lower FCR than Deshi, while differences with Sonali were not significant.

Deshi chickens exhibited moderate and persistent infection patterns. No clinical signs or oocyst shedding were observed at 6 DPI. At 10 DPI, oocyst shedding increased, 1700 ± 175.71 OPG accompanied by mild

lesions (score 1) and a significant reduction in weight gain (80 ± 5.27 g) compared to other infected breeds. By 14 DPI, weight gain increased to 123 ± 3.61 g, remaining intermediate among the infected groups, while FCR remained high throughout the infection period (4.83 ± 0.33 at 10 DPI and 4.59 ± 0.13 at 14 DPI), significantly higher than some breeds but showing no further significant changes over time.

3.2. Histopathological finding

Histopathological examination of the cecum at 10 days post-infection (DPI) with *E. tenella* revealed marked differences in tissue response among various chicken breeds (Fig. 1). In the uninfected Sonali chickens, the cecal mucosa displayed normal architecture with intact villi and no signs of cellular disruption, serving as the negative control for comparison. In contrast, Sonali chickens challenged with *E. tenella* showed severe necrosis of the intestinal villi and accumulation of necrotic debris in the lumen, indicating extensive epithelial damage and mucosal compromise.

Deshi chickens exhibited desquamation of epithelial enterocytes lining the villi, reflecting a moderate level of infection-induced injury. The Naked Neck breed showed the most profound pathology, including total villous desquamation and widespread epithelial loss, suggesting a heightened susceptibility to coccidia infection or a more severe inflammatory response. Conversely, Hilly chickens showed only mild histopathological alterations, including partial desquamation and minor villous distortion, indicating a relatively lower level of susceptibility or greater resilience to *E. tenella* infection.

These breed-specific differences in cecal tissue integrity suggest that genetic or immunological factors may influence the severity of coccidial infections. The extensive villous necrosis observed in Naked Neck and Sonali chickens may impair nutrient absorption and overall gut function, thereby affecting growth and productivity. The relatively preserved mucosal architecture in Hilly chickens supports the hypothesis that certain indigenous breeds may possess inherent resistance to *E. tenella*. Further research into host immune responses and genetic

markers of resistance may provide insights for breeding and disease management strategies in poultry farming.

4. Discussion

In the present study, a relatively high challenge dose of 70,000 sporulated *Eimeria tenella* oocysts was used to induce consistent pathological lesions and stimulate measurable immune responses, particularly in native chicken breeds. This dose selection was intentional, as indigenous chickens such as Naked Neck, Deshi, and Hilly types are often considered more resistant or tolerant to various pathogens, including *Eimeria* species, compared to commercial hybrids (Pinard-Van Der Laan et al., 1998). Lower doses ranging from 4000 to 12,000 oocysts have been reported as sufficient to produce lesions in highly susceptible birds such as commercial layer and hybrid chickens (Soutter et al., 2021). For instance, Soutter et al. (2021) used doses from 5000 to 50,000 oocysts and demonstrated dose-dependent effects on lesion scores and cytokine responses in different layer breeds. Similarly, Melkamu et al. (2017) employed 20,000 oocysts to induce infection in broiler chickens, while Teng et al. (2020) used 50,000 oocysts and showed that higher doses linearly regulated gene expression of tight junction proteins, affected growth performance, and intestinal morphology in broilers. Kurkure et al. (2006) used a dose rate of 50,000 oocysts to evaluate polyherbal herbal mixture against *E. tenella* infection in broiler with OPG, histopathology, clinical signs, lesion scores and immunological characteristics (humoral and cell mediated immune response). Notably, Pinard-Van Der Laan et al. (1998) challenged 4-week-old outbred chickens with a very high dose of 150,000 oocysts to evaluate genetic resistance to *E. tenella*. These reports collectively support the rationale for using a 70,000-oocyst challenge in our study, as it falls within the upper-middle range of previously validated infection models and is appropriate for detecting both immunological and pathological responses in native breeds presumed to have greater resistance. This higher dose ensures adequate parasite replication and lesion development for comparative assessment of resistance and performance traits across breeds.

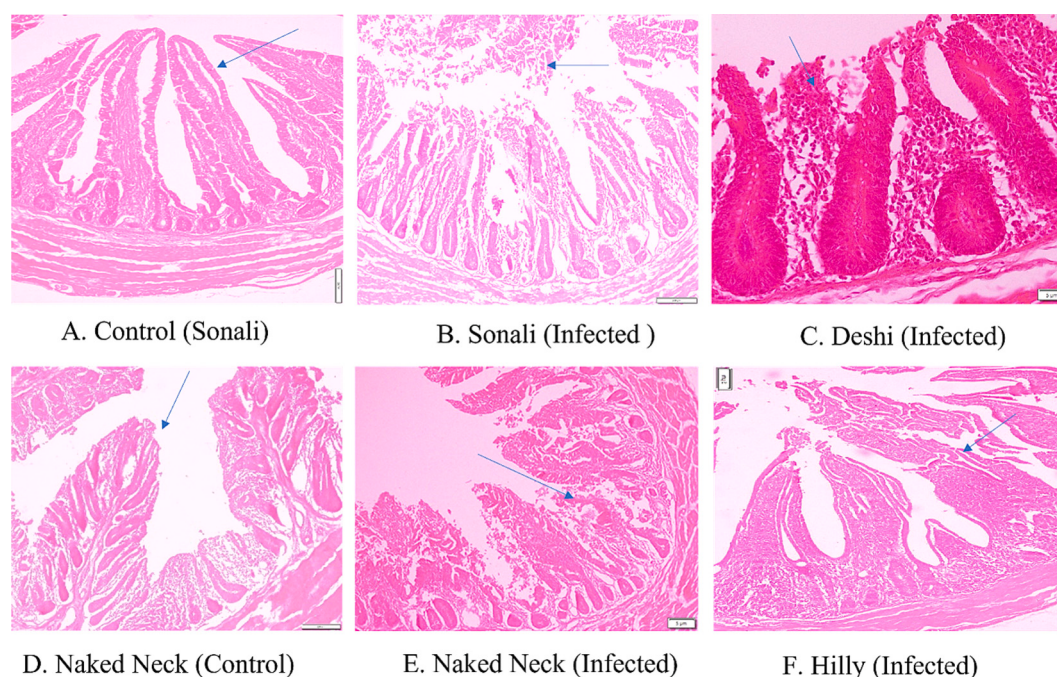


Fig. 1. Photomicrographs of the cecum from different breeds of chickens at 10 days post-infection (DPI). Arrows indicate pathological changes. (A) Uninfected Sonali chicken showing intact and normal villi. (B) Infected Sonali chicken exhibiting severe necrosis of intestinal villi and presence of necrotic debris in the intestinal lumen. (C) Infected Deshi chicken showing desquamation of epithelial enterocytes lining the villi. (D) Uninfected Naked Neck chicken with normal villous architecture. (E) Infected Naked Neck chicken demonstrating marked desquamation and damage of villi and epithelial tissue. (F) Infected Hilly chicken showing comparatively less desquamation and milder villous damage.

4.1. Sonali chickens

Sonali chickens, a commercial crossbreed widely used in Bangladesh, exhibited the highest susceptibility to *Eimeria tenella* infection in this study. Infected Sonali birds showed a marked reduction in body weight gain (BWG), elevated feed conversion ratio (FCR), severe bloody diarrhea, and pronounced lethargy. Oocyst shedding was significantly higher in this group, reaching 103,200 oocysts per gram (OPG) at 14 days post-infection (DPI), with corresponding qPCR results confirming the highest gene copy number ($8.11 \pm 0 \log_{10}$ copies/g). Histopathologically, Sonali birds presented with extensive cecal necrosis, villous atrophy, epithelial desquamation, and hemorrhagic lesions. These findings align with the observations by (Jatau et al., 2014) and (Blake et al., 2020), who reported that broiler and commercial hybrid birds are typically more vulnerable to coccidiosis due to their selection for rapid growth rather than immunological robustness. The pronounced clinical and pathological manifestations observed in Sonali chickens suggest a high pathogenic burden and limited innate resistance, indicating the need for enhanced prophylactic interventions, such as vaccination and nutritional support, in commercial production systems relying on this breed.

4.2. Naked neck chickens

Naked Neck chickens demonstrated intermediate resistance to *E. tenella* infection. Despite infection, Naked Neck chickens recorded relatively higher weight gain among infected breeds and a moderate FCR, suggesting a capacity for recovery after initial infection (Table 1). Oocyst shedding was moderate, and the qPCR-detected parasite gene copy number was significantly lower than that of Sonali birds. Histopathological analysis revealed localized cecal inflammation and epithelial disruption, but with relatively preserved mucosal architecture compared to Sonali chickens. The moderate response is consistent with findings by Ferdushy et al. (2016) and (Hemanth et al., 2024), who noted that Naked Neck chickens possess heat tolerance and a degree of disease resilience, possibly linked to genetic adaptations favoring better gut health and immune modulation. Although not as resistant as other native breeds in this study, Naked Neck chickens still represent a promising dual-purpose genotype with moderate tolerance to coccidial infections, suggesting that selective breeding could further enhance their resilience.

4.3. Hilly chickens

Hilly chickens exhibited the highest resistance among the studied breeds. Infected Hilly birds maintained relatively stable BWG and FCR, with low clinical signs and negligible diarrhea. Oocyst output was the lowest across all groups (100 OPG at 14 DPI), and qPCR quantification confirmed the lowest gene copy numbers, indicating minimal parasite replication. Cecal tissues showed minor to no histopathological alterations, with preserved mucosal integrity and negligible inflammation. These findings are strongly supported by studies such as those by (Choi et al., 2021) who reported enhanced immune responses and disease tolerance in native or local ecotypes exposed to coccidial pathogens. The robust performance of Hilly chickens in the face of *E. tenella* challenge highlights their potential value for sustainable, low-input production systems where coccidiosis remains a major constraint. Their genetic resistance may be associated with better mucosal immunity, local antigen presentation, and gut microbiota stability, all of which warrant further investigation.

4.4. Deshi (non descriptive) chickens

Deshi chickens, widely recognized for their hardiness and adaptability, showed moderate resistance to *E. tenella* infection. Although they had a relatively higher FCR compared to other breeds likely due to

inherently slower growth they demonstrated minimal BWG reduction and less severe clinical signs. Oocyst shedding was moderate, and qPCR data revealed intermediate gene copy numbers. Histological evaluation showed mild cecal damage, characterized by focal epithelial erosion and light lymphocyte infiltration. Earlier studies, including those by Islam and Nishibori (2009) and Ferdushy et al. (2016), have documented the resilience of native Bangladeshi chickens to parasitic infections, attributing it to their natural exposure to environmental pathogens and resulting immune adaptation. The ability of Deshi birds to withstand coccidial infection with minimal productivity loss further supports their role in backyard and small-scale systems, where therapeutic interventions may be limited. However, their low feed efficiency and production potential remain a challenge for commercial scaling.

Overall, Sonali chickens, representing an exotic breed, were the most susceptible to *E. tenella* infection, with severe clinical and performance impairments. In contrast, the native breeds, especially Hilly and Naked Neck, exhibited greater resilience marked by lower oocyst shedding, milder lesions, and sustained growth performance. Statistical analysis further confirmed breed-specific variations in FCR, particularly for Sonali. Although lesion scores across breeds did not reach statistical significance, comparisons between Sonali and the native breeds (Hilly and Naked Neck) approached significance ($p = 0.0513$), supporting observed trends in resistance.

These findings highlight substantial variation in the response to *E. tenella* infection among breeds, underscoring the potential of native chickens for improved resilience through genetic selection or breeding programs.

5. Conclusion

The comparative analysis of breed-specific responses clearly indicates that native and regionally adapted chickens such as Hilly and Deshi are more resilient to *E. tenella* infection than commercial types like Sonali. Naked Neck chickens fall in between, showing moderate tolerance. These differences emphasize the importance of genetic background in disease resistance and support the integration of indigenous breeds into poultry development programs aimed at improving sustainability, reducing drug dependency, and enhancing food safety.

CRedit authorship contribution statement

Dipesh Aryal: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Kazi Farah Tasfia:** Visualization, Methodology, Investigation, Formal analysis. **Al Nur Tarak:** Methodology, Investigation, Data curation. **Asmita Bhujel:** Validation, Methodology, Data curation. **Md. Shahiduzzaman:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Ethical approval

Chickens were handled following the guidelines approved by the Animal Welfare and Experimentation Ethics Committee of Bangladesh Agricultural University, Mymensingh (Approval number: AWEEC/BAU/2018).

Ethical statement

The experimental infection and all handling of chickens in this study were performed following the ethical guidelines for animal research and were approved by the Animal Welfare and Experimentation Ethics Committee of Bangladesh Agricultural University (Approval No.: AWEEC/BAU/2023-45). The birds were monitored daily, and all efforts were made to minimize discomfort and distress. Humane endpoints were applied, and no unnecessary suffering occurred during the study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the reported in this article.

Acknowledgments

The authors would like to express their sincere gratitude to Dr. Shakila Faruque, Bangladesh Livestock Research Institute (BLRI) for providing pure breeds of native chickens (Hilly, Naked Neck chicks) for this study. The authors also acknowledge Mr. Md. Ashfaq Sadat for his assistance with the statistical analysis. This work was supported by the Bangladesh Academy of Science [BAS-USDA Program/2023/26(12)].

References

- Attia, M.M., Mohamed, R.I., Salem, H.M., 2023. Impact of *Eimeria tenella* experimental infection on intestinal and splenic reaction of broiler chickens. *J. Parasit. Dis.* 47 (4), 829–836. <https://doi.org/10.1007/s12639-023-01629-z>.
- Barua, A., Howlider, M.A.R., Yoshimura, Y., 1998. Indigenous naked neck fowl of Bangladesh. *Worlds Poult. Sci. J.* 54 (3), 279–286. <https://doi.org/10.1079/WPS19980019>.
- Blake, D.P., Qin, Z., Cai, J., Smith, A.L., 2008. Development and validation of real-time polymerase chain reaction assays specific to four species of *Eimeria*. *Avian Pathol.* 37 (1), 89–94. <https://doi.org/10.1080/03079450701802248>.
- Blake, D.P., Knox, J., Dehaeck, B., Huntington, B., Rathinam, T., Ravipati, V., Ayoade, S., Gilbert, W., Adebambo, A.O., Jatau, I.D., Raman, M., Parker, D., Rushton, J., Tomley, F.M., 2020. Re-calculating the cost of coccidiosis in chickens. *Vet. Res.* 51 (1), 115. <https://doi.org/10.1186/s13567-020-00837-2>.
- Choi, J., Ko, H., Tompkins, Y.H., Teng, P.-Y., Lourenco, J.M., Callaway, T.R., Kim, W.K., 2021. Effects of *Eimeria tenella* infection on key parameters for feed efficiency in broiler chickens. *Animals* 11 (12), 3428. <https://doi.org/10.3390/ani11123428>.
- Conway, D.P., McKenzie, M.E., 2007. *Poultry Coccidiosis*, 3rd ed. Wiley. <https://doi.org/10.1002/9780470344620>.
- Dalloul, R.A., Lillehoj, H.S., 2006. Poultry coccidiosis: recent advancements in control measures and vaccine development. *Expert Rev. Vaccines* 5 (1), 143–163. <https://doi.org/10.1586/14760584.5.1.143>.
- Ferdus, T., Hasan, M.T., Golam Kadir, A.K., 2016. Cross sectional epidemiological investigation on the prevalence of gastrointestinal helminths in free range chickens in Narsingdi district, Bangladesh. *J. Parasit. Dis.* 40 (3), 818–822. <https://doi.org/10.1007/s12639-014-0585-5>.
- Habibi, H., Firouzi, S., Nili, H., Razavi, M., Asadi, S.L., Daneshi, S., 2016. Anticoccidial effects of herbal extracts on *Eimeria tenella* infection in broiler chickens: in vitro and in vivo study. *J. Parasit. Dis.* 40 (2), 401–407. <https://doi.org/10.1007/s12639-014-0517-4>.
- Hemanth, M., Venugopal, S., Devaraj, C., Shashank, C.G., Ponnuvel, P., Mandal, P.K., Sejian, V., 2024. Comparative assessment of climate resilient potential in four poultry genotypes reared in hot-humid tropical environment: a preliminary evaluation. *Int. J. Biometeorol.* 68 (11), 2267–2279. <https://doi.org/10.1007/s00484-024-02744-z>.
- Henriksen, S.A., Aagaard, K., 1976. A simple flotation and McMaster method (author's transl). *Nord. Vet. Med.* 28 (7–8), 392–397.
- Holdsworth, P.A., Conway, D.P., McKenzie, M.E., Dayton, A.D., Chapman, H.D., Mathis, G.F., Skinner, J.T., Mundt, H.-C., Williams, R.B., 2004. World Association for the Advancement of Veterinary parasitology (WAAVP) guidelines for evaluating the efficacy of anticoccidial drugs in chickens and turkeys. *Vet. Parasitol.* 121 (3–4), 189–212. <https://doi.org/10.1016/j.vetpar.2004.03.006>.
- Islam, M.A., Nishibori, M., 2009. Indigenous naked neck chicken: a valuable genetic resource for Bangladesh. *Worlds Poult. Sci. J.* 65 (1), 125–138. <https://doi.org/10.1017/S0043933909000105>.
- Jatau, I.D., Odika, A.N., Thlana, M., Talba, A.M., Bisalla, M., Musa, I.W., 2014. Response of 2 breeds of broiler chicks to experimental infection with low dose of *Eimeria tenella* sporulated oocysts. *Turk. J. Vet. Anim. Sci.* 38, 398–404. <https://doi.org/10.3906/vet-1306-22>.
- Johnson, J., Reid, W.M., 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. *Exp. Parasitol.* 28 (1), 30–36. [https://doi.org/10.1016/0014-4894\(70\)90063-9](https://doi.org/10.1016/0014-4894(70)90063-9).
- Kurkure, N.V., Kolte, S.W., Bhandarkar, A.G., Kalorey, D.R., 2006. Evaluation of herbal coccidiostat “Coxynil” in broiler. *Indian J. Exp. Biol.* 44 (9), 740–744.
- Lillie, R., 1965. *Histopathologic Technique and Practical Histochemistry*. McGraw Hill Book Company, New York.
- Melkamu, S., Chanie, M., Asrat, M., 2017. Studies on Coccidia in experimental infection with *Eimeria* spp in rose-cobb broiler chicken. *J. Anim. Res.* 7 (1), 115. <https://doi.org/10.5958/2277-940X.2017.00016.X>.
- Monira, K., Hussain, S., 2020. Phenotypic characteristics of three indigenous chicken genotypes in Bangladesh. *Bangladesh J. Livestock Res.* 10–23. <https://doi.org/10.3329/bjlr.v0i0.45442>.
- Pinard-Van Der Laan, M.H., Monvoisin, J.L., Pery, P., Hamet, N., Thomas, M., 1998. Comparison of outbred lines of chickens for resistance to experimental infection with coccidiosis (*Eimeria tenella*). *Poult. Sci.* 77 (2), 185–191. <https://doi.org/10.1093/ps/77.2.185>.
- Rabbani, M.A.G., Vallejo-Trujillo, A., Wu, Z., Miedzinska, K., Faruque, S., Watson, K.A., Smith, J., 2024. Whole genome sequencing of three native chicken varieties (common Deshi, hilly and naked neck) of Bangladesh. *Sci. Data* 11 (1), 1432. <https://doi.org/10.1038/s41597-024-04291-z>.
- Raha, S.K., 2007. Poultry industry in Bangladesh: is it growing? *Bangladesh J. Agric. Econ.* 30 (2), 93–101.
- Sagolsem, S., Singh, Y.D., Rajkhowa, T.K., Ravindran, R., Arya, R.S., Patra, G., Kalita, A., 2021. Clinico-pathomorphological studies and diagnosis of caecal coccidiosis in chicken population of Mizoram, India. *Indian J. Vet. Pathol.* 45 (1), 53–55. <https://doi.org/10.5958/0973-970X.2021.00008.0>.
- Soutter, F., Werling, D., Kim, S., Pastor-Fernández, I., Marugán-Hernández, V., Tomley, F.M., Blake, D.P., 2021. Impact of *Eimeria tenella* oocyst dose on parasite replication, lesion score and cytokine transcription in the caeca in three breeds of commercial layer chickens. *Front. Vet. Sci.* 8. <https://doi.org/10.3389/fvets.2021.640041>.
- Teng, P.-Y., Yadav, S., Castro, F.L.S., Tompkins, Y.H., Fuller, A.L., Kim, W.K., 2020. Graded *Eimeria* challenge linearly regulated growth performance, dynamic change of gastrointestinal permeability, apparent ileal digestibility, intestinal morphology, and tight junctions of broiler chickens. *Poult. Sci.* 99 (9), 4203–4216. <https://doi.org/10.1016/j.psj.2020.04.031>.
- Uddin, M.H., A. A., A. Y., K. M.A., 2011. Geographical distribution, classification, characterization and conservation of different native chicken varieties of Bangladesh. *Bangladesh Res. Publ. J.* 5 (3), 227–233.
- Yeasmin, T., Howlider, M.A.R., 1998. Comparative physical features, egg production and egg quality characteristics of normal and dwarf indigenous (*Deshi*) hens of Bangladesh. *J. Appl. Anim. Res.* 13 (1–2), 191–196. <https://doi.org/10.1080/09712119.1998.9706686>.