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Epidemiology and Microscopic Characterization of Eimeria Species from Chickens in District Sialkot, Pakistan

Asma Waheed Qureshi[1*] and Meerub Sarfraz[1]

[1]Department of Zoology, Government College Women University, Sialkot, Pakistan

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***Corresponding Author:**
Asma Waheed Qureshi
Department of Zoology, Government College Women University, Sialkot, Pakistan
asma.qureshi@gcwus.edu.pk

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ABSTRACT

Coccidiosis is an illness that affects poultry, leading to huge economic losses, and is exhibited by protozoans belonging to the genus *Eimeria*. **Objectives:** The study was designed to identify the incidence of coccidiosis in the District Sialkot chicken and *Eimeria* species, as well as to determine the relationship between age and seasonal change. **Methods:** 250 fecal samples were obtained in the period between August 2019 and January 2020. The samples were treated by the methods of sedimentation and flotation. Chi-square and correlation were used to test associations between risk factors and disease events. **Results:** 250 samples were positive, with 117 (46.8) samples containing *Eimeria* oocysts. The prevalence was 45, 52.72, 60, 42, 36, and 20 during August to January, with the highest prevalence in October and the lowest prevalence being in January. The rate of infection was more significant in 13-month-olds (56.12) than that of 5 to 6 months (46.59) and 13 months or more (32.81), with a significant age correlation (P = 0.01). Five species of *Eimeria* were identified: *E. maxima, E. acervulina, E. mitis, E. tenella, and E. necatrix*. **Conclusions:** The prevalence of coccidiosis is much higher in District Sialkot, particularly in young chickens and peak months. The results are valuable epidemiological insights that could be used in prevention and control measures to reduce economic costs.

INTRODUCTION

Poultry farming is among the most developed industries of the globe [1]. Over the last decade, it has grown very speedily in Pakistan [2]. But poultry production has been susceptible to various dangers, including bacterial, viral, and parasitic diseases. Out of the parasitic diseases, the major obstacle in poultry development is coccidiosis [1]. This disease is caused by obligate intracellular protozoa belonging to the *Eimeria genus* [3]. Seven infective *Eimeria* species include: *E. tenella*, *E. acervulina*, *E. mitis*, *E. necatrix*, *E. maxima*, *E. brunetti*, and *E. praecox*. These Eimeria species target particular regions of the GIT of birds [4]. In poultry, Coccidiosis exists in two forms: caecal and intestinal [5]. *E. tenella* causes caecal coccidiosis because it infects the caeca. Intestinal coccidiosis, which is a chronic form of disease, is caused by *E. maxima* and *E. acervullina* [6]. Coccidiosis is generally marked by bloody diarrhea, inflammation of the intestine, malabsorption, decreased growth, and ruffled feathers [7]. This disease is prevalent in the tropics and subtropics. The ecological conditions and management in these regions support and promote the development and transmission of *Eimeria* throughout the year [8]. The distribution and prevalence of coccidiosis are influenced by multiple factors. These factors include: high animal density in a small space, high air temperature and high relative humidity, different age groups of birds at the same place, feed change, and health condition of the bird [9]. This parasitic disease causes great financial damage in the production of poultry

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products worldwide [10]. To control coccidiosis, the poultry industry commonly uses three main approaches: anticoccidial drugs, vaccination, and improving management practices. The identification of different *Eimeria* species also plays a very important role in effective control and in disease management strategies. Although several studies have reported the prevalence of coccidiosis in different regions of Pakistan, district-level data from Sialkot are scarce, and the circulating *Eimeria* species in this area have not been adequately documented using microscopic characterization [11]. Moreover, existing studies largely focus on prevalence without providing localized baseline data to support region-specific control strategies. This lack of area-specific prevalence and species identification data constitutes an important knowledge gap, which the present study aims to address. Researchers have conducted studies on coccidiosis across Pakistan, yet further investigation into its epidemiological aspects is needed to improve disease control and poultry farming productivity. District Sialkot is a major poultry-producing region in Punjab, with a high density of commercial and backyard farms, yet recent and comprehensive data on coccidiosis prevalence and species distribution in this area are lacking.

The main aim of this study was to address the regional knowledge gap in District Sialkot by investigating the prevalence and species identification of *Eimeria* in chickens and assessing the association of coccidiosis with various risk factors.

METHODS

A cross-sectional study was conducted over six months from August 2019 to January 2020 to determine the prevalence and identify the species of *Eimeria* in chickens in District Sialkot, Punjab, Pakistan. Fecal samples were collected from chickens on poultry farms located in multiple villages across the district (Ahmal Pur, Saidpur, Baddiana, Ballanwala, Bajwat, Bhagowal, Daska, Dallowali, Meradke, Mukta, and Zafarwal). The study was conducted in Sialkot, which is one of the districts of the Punjab province of Pakistan. Fecal samples were collected using a convenience sampling technique from accessible poultry farms in District Sialkot during the study period. Due to logistical constraints, random farm selection was not feasible; therefore, the findings may not be fully representative of all poultry farms in the district Baddiana, Ballanwala, Bajwat, Bhagowal, Daska, Dallowali, Meradke, Mukta, and Zafarwal) of Sialkot. The sample size was calculated using the standard formula for estimating prevalence: $n = Z^2 \times p \times (1-p)/d^2$, where Z = 1.96 corresponds to a 95% confidence level, p = 0.5 (assumed prevalence in the absence of prior district-level data), and d=0.06 represents the desired margin of error. This yielded a minimum required sample size of 267. Due to logistical constraints, 250 samples were collected, which was considered adequate to provide a reasonable estimate of prevalence. A total of 250 samples were taken from three different age groups (1-3 months, 5-6 months, and 1year and above) of hens and cocks/ during a period from August, 2019 to January, 2020. Chickens were categorized into three age groups: 1–3 months, 5–6 months, and ≥1 year. These groupings were based on commonly recognized poultry production stages, representing early growth, grower phase, and mature birds, respectively. Although the intervals are uneven, they reflect biologically relevant stages of immunity development and management practices. This categorization may introduce some degree of misclassification bias, which is acknowledged as a study limitation. Freshly deposited feces were collected in polythene zipper bags and stored at 4 °C until further processing. During the sampling period average temperature ranged between 35 °C and 15 °C. The average humidity during the sampling period ranged between 59% and 40%. A convenience sampling strategy was employed across 14 poultry farms in the selected villages of District Sialkot. The sample size of 250 was calculated using the expected prevalence formula for cross-sectional studies. A Foundation for Analysis in the Health Sciences. based on an estimated prevalence of 50% (to maximize sample size), a 95% confidence level, and a 5% margin of error. A total of 250 fresh fecal samples were collected from chickens belonging to three age categories: young chicks (1–3 months), growers (5–6 months), and adults (≥1 year). These categories were chosen to reflect distinct physiological and immunological stages relevant to coccidiosis susceptibility, with the first two intervals focusing on periods of highest known vulnerability. Sampling was conducted during the period from August 2019 to January 2020. Freshly deposited feces were collected in sterile polythene bags and stored at 4°C until processing. Samples were initially processed by the dilution method and straining to remove the debris. After the straining process, the strained fecal sample was placed at bench top and left for 10-15 minutes for the sedimentation of oocysts. Then samples were examined under a microscope to detect the presence of oocysts in feces. From the positive samples, non-sporulated oocysts (Figure 1A) were isolated by using the saturated saline flotation and centrifugation method. Isolated oocysts were then sporulated by placing them in a solution of 2.5% potassium dichromate (Sigma-Aldrich, Germany) at room temperature for seven days [12]. Then, sporulated oocysts (Figure 1B) were stored at 4 °C.

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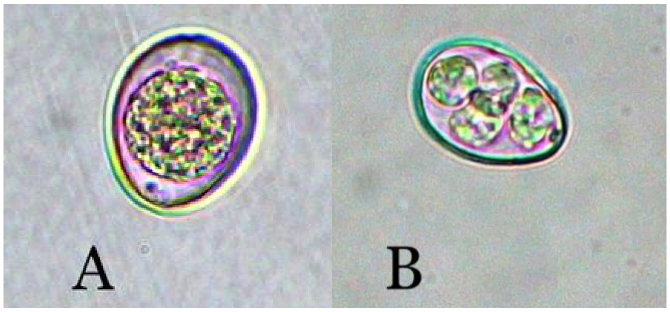

Figure 1: (A) Nonsporulated Oocyst (B) Sporulated Oocyst

Sporulated oocysts were examined under a microscope (Biological Compound Microscope, Model CXL, Labo America Inc., USA) at 40X magnification for species identification based on morphological characteristics. For the quantification of oocyst output, a McMaster counting chamber was used. Briefly, a 3 g aliquot of each positive fecal sample was thoroughly mixed with 42 mL of saturated salt solution. The mixture was filtered through a sieve, and the chamber was filled. Oocysts in both chambers were counted under 100X magnification, and the number of oocysts per gram of feces (OPG) was calculated using the formula: OPG = (Total oocyst count × 100) / 3. For each sample, quantification was performed in duplicate, and the average was recorded. For species identification, 50 intact oocysts per positive sample were measured (length and width) using ImageJ software (Java 1.8.0-172), and the shape index (length/width) was determined. The data was analyzed to determine the percentage prevalence. It was calculated by dividing No. of positive samples by total No. of samples and multiplying by 100. For statistical analysis, IBM SPSS version 23.0 was used. Chi-square analysis was used to determine the association between categorical variables and the occurrence of coccidiosis. The p-values less than 0.05 were considered significant.

RESULTS

Out of 250 samples, 117 were found to be positive. The overall prevalence of infection was 46.8% (117/250), with a 95% confidence interval (CI) of 40.6%-53.0%. The lowest prevalence (%) was observed during January, while the highest positive cases were found in the month of October. Prevalence (%) in each sampling month is shown in table 1.

Table 1: Month-Wise Positive Cases and Prevalence (%) in Each Month

Month of Year	Total No. of Samples	Positive Samples	Prevalence (%)
August	40	18	45
September	55	29	52.72
October	60	36	60
November	50	21	42
December	25	09	36
January	20	04	20
Total	250	117	46.8

The prevalence of infection was highest (56.12 %, 55/98) in 1-3-month-old chickens. In the case of the 5-6 months age group, prevalence was 46.6% (41/88). The lowest rate of disease, 32.81% (21/64), was found in the 1 year and above age group (Figure 2).

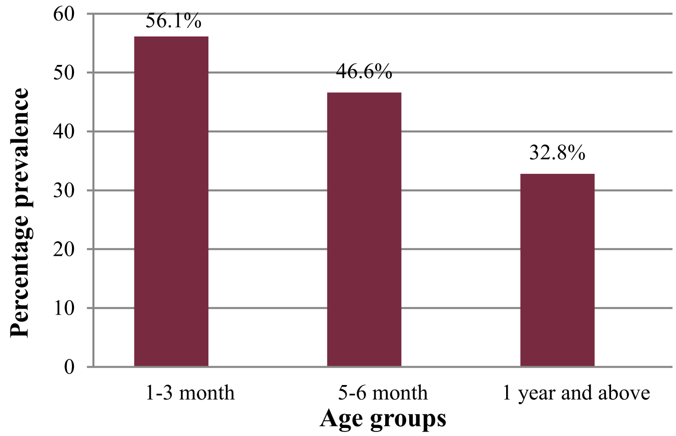

Figure 2: Overall Percentage Prevalence in Each Age Group of Chickens

A total of 123 samples were taken from cocks. Out of these, 53 (43.08%) samples were recorded as positive. In the case of hens, 64 (50.39%) were positive out of 127 samples. The rate of coccidiosis was higher in female than in male (Figure 3).

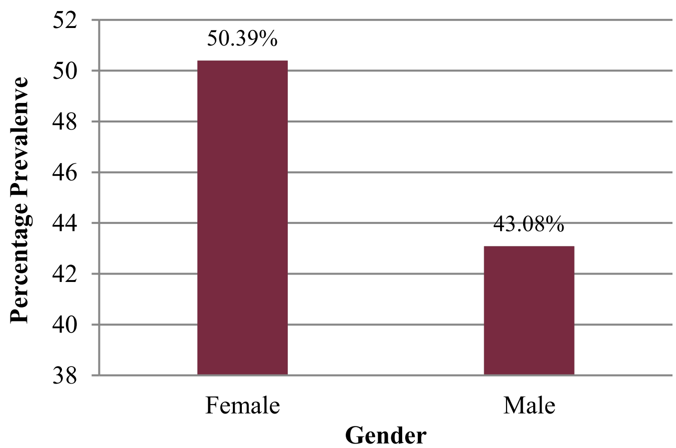

Figure 3: Overall Percentage Prevalence in Female and Male

Results of Chi square revealed that there was a non-significant relation between gender and prevalence of disease. Significant ($P < 0.05$) association was observed between age and disease, and temperature and occurrence of disease (Table 2).

Table 2: Association of Various Factors with the Occurrence of Disease

Risk Factors	Total Samples	Positive Cases	Prevalence (%)	χ^2	p-value
Gender					
Male	123	53	43.08	1.33	0.24
Female	127	64	50.39		

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Age Group					
1-3 months	98	55	56.12	8.45	0.01
5-6 months	88	41	46.59		
≥1 year	64	21	32.81		
Months (Aug-Jan)	250	117	46.80	—	0.03

$p<0.05$ = significant; $p>0.05$ = non-significant; χ^2 = Chi square statistic

On the basis of morphological characters, five species of *Eimeria* were identified from chickens in Sialkot. ImageJ software (Java 1.8.0-172) was used for the quantification of the length and width of sporulated oocysts (Table 3).

Table 3: Measurements of Oocysts and Identified Species

Length of Oocysts (in um)	Width of Oocysts (in um)	Shape Index (Length /Width)	Identified Species
Mean ± SD			
29.55 ± 0.391	20.91 ± 0.499	1.41	*E. maxima*
17.85± 0.896	13.98 ± 0.331	1.28	*E. acervullina*
15.04 ± 0.295	13.75 ± 0.618	1.09	*E. mitis*
23.36 ± 0.684	18.81 ± 0.621	1.24	*E. tenella*
19.59 ± 2.183	16.00 ± 1.787	1.22	*E. necatrix*

SD = Standard deviation

Five different species were identified in *Eimeria*, like (A) *E. maxima*, (B) *E. acervulina*, © *E. mitis*, (D) *E. tenella*, and (E) *E. necatrix* (Figure 4).

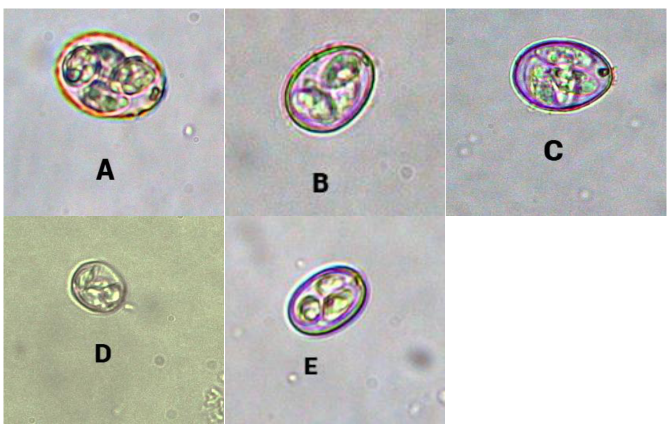

Figure 4: Identified species of *Eimeria*: **A:** *E. maxima*, **B:** *E. acervulina*, **C:** *E. mitis*, **D:** *E. tenella*, **E:** *E. necatrix*

DISCUSSION

Coccidiosis is one of the noteworthy diseases from an economic and medical point of view. This infection of *Eimerian* species is almost present in all regions of the globe [12]. Studies based on investigating the occurrence and prevalence of diseases play an important role in the control of disease. In the present study, the occurrence of coccidian species in chickens from District Sialkot was investigated. Results of this current study indicated that the disease was present in all six months from August to January. A significant association (p=0.03) was found between the month (temperature) and the occurrence of disease. The overall prevalence of disease in this study is 46.8%. The report of Jamil *et al.* was comparable with the present finding, which reported 44% prevalence from D.I.K (Pakistan) [1]. Several previous reports showed a lower rate of prevalence in different regions of Pakistan. Shamim *et al.* accounted 9.59 % prevalence in Mirpur (Azad Kashmir) [5]. Amin *et al.* reported the prevalence as 37.91% in Abbottabad (Pakistan) [13]. Another report by Sohail *et al.* recorded 10% total occurrence in Abbottabad (K.P.K). Some other findings also showed a higher rate of occurrence in different regions of Pakistan than the results of this study [14]. Khan reported 58% prevalence in Southern Punjab [15]. In the District of Gujrat (Pakistan), Naveed and Faryal documented 67.57% of the disease [16]. Bachaya *et al.* in Muzaffargarh (Pakistan) reported 65% of overall rate of infection [2]. This difference in the incidence of disease may be due to differences in weather conditions, studies conducted in different months, seasons of the year, and different conditions of management at farms in disparate regions of Pakistan. Maximum prevalence of disease was recorded in October (60%), followed by September (52.72%). The reason of high cases in these months may be because of favorable temperature and relatively high humidity, which are suitable conditions for the sporulation and then spread of disease. In this study, a decrease in positive cases was observed in the month of November, which may be due to the drop in temperature and relative humidity in this month. The least prevalence was observed in December. The outcomes of the study revealed that appropriate temperature and high relative humidity in the atmosphere may lead to an increase in the incidence of disease. This outcome of the study aligns with findings of Bachaya *et al.* who reported the highest cases in month of September and October in Southern Punjab [2]. Ali *et al.* also recorded the higher infection in October in Quetta (Pakistan) [10]. In contrast, Amin *et al.* and Awais *et al.* reported the highest occurrence of disease in August and September in Abbottabad and Rawalpindi (Pakistan) Respectively [13, 17]. Age-wise findings revealed that there was a significant relationship between the disease and the age of chicks. It was found that the rate of disease was higher in the chicks of the 1-3 months age group, followed by 5-6 months old chicks. The lowest prevalence was found in the 1-year-and-above age group of birds. These results are in harmony with findings of Bachaya *et al.* and Mahboob *et al.* who reported that the infection was more common in younger chickens in comparison to adults [2, 18]. A feasible reason for the higher rate of prevalence of coccidian infection in the 1–3-month age group of chicks may be that the chicks having an age between 31days to 45 have not developed immunity to resist the infection. This may lead to higher cases of disease in this age. As the chicks grow and become older, they acquire immunity to resist the infection [13]. This fact explains the lower rate of disease in older age

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chickens. Analysis of gender wise prevalence indicated a non-significant relation between gender and prevalence of disease. The rate of disease was higher in female chickens than in male chickens. These findings correlate with the results of Ketema and Fasil, who reported a higher rate of infection in female (20.1%) as compared to male (18.5%) [19]. Contrary to current findings, Alemayehu *et al.* observed a higher frequency of disease in male than female in Addis Ababa, Ethiopia [20]. In the current study, five species of *Eimeria* were identified primarily based on oocyst morphology (size, shape index, and color). *E. necatrix* oocysts were oblong, and *E. maxima* oocysts were the largest and exhibited a distinctive yellowish color [11]. Among the identified species, *E. tenella* is the most pathogenic species. It affects the ceca of birds, so it is responsible for the caecal coccidiosis. Other recognized species are responsible for intestinal coccidiosis. *E. necatrix* and *E. acervulina* are also highly pathogenic. *E. maxima* show moderate pathogenic effects while *E. mitis* has low pathogenicity. Previous reports by different researchers in different regions of Pakistan reported the presence of various species in chickens. The findings of Jamil *et al.* were in line with the present study, which reported the occurrence of *E. mitis*, *E. maxima*, *E. tenella*, and *E. necatrix* in D.I.K (Pakistan). The findings of Sohail *et al.* were partially in harmony with current results, which reported the occurrence of *E. mitis*, *E. tenella*, *E. maxima*, and *E. acervulina* in Khyber-Pakhtunkhwa [14]. Awais *et al.* documented the existence of *E. tenella* and *E. maxima*, *E. necatrix*, and *E. acervulina* in Faisalabad. Two species of Eimeria i.e *E. maxima* and *E. tenella*, were reported in Azad Kashmir by Shamim *et al.* [5]

CONCLUSIONS

The results showed that coccidiosis is moderately prevalent in District Sialkot. Both intestinal and caecal forms of the disease were confirmed to be present. The study identified that younger age, warmer temperatures, and higher humidity levels were significantly associated with a higher prevalence of *Eimeria* infection. These findings highlight important factors to consider for targeted management. The data generated by this research can inform regional control strategies, suggesting that heightened biosecurity and prophylactic measures are particularly warranted for young flocks during warm, humid periods. Future longitudinal studies are recommended to establish causal relationships and quantify the economic impact of these associations.

Authors Contribution

Conceptualization: AWQ
Methodology: MS
Formal analysis: AWQ, MS
Writing and Drafting: AWQ, MS
Review and Editing: AWQ, MS

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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