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

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ABSTRACT

Coccidiosis is a disease of poultry that causes severe economic losses to the poultry farmers. This disease is caused by the protozoan parasite belonging to the genus *Eimeria*. These parasites are prevalent in chickens in the Sialkot district. **Objectives:** To investigate the prevalence of coccidiosis in chickens from District Sialkot and the microscopic identification of parasite species causing this infection. **Methods:** For this purpose, a total of 250 fecal samples were collected randomly from chickens from August, 2019 to January, 2020. Samples were analyzed by microscopy, and positive samples were processed by the sedimentation and flotation technique to isolate oocysts for identification. Chi-square was used to determine the relation of various factors to the disease. **Results:** Out of 250 samples, 117 were found positive for oocysts of *Eimeria*. Month-wise prevalence of disease was recorded as 45%, 52.72%, 60%, 42%, 36% and 20% during August, September, October, November, December, and January, respectively. The rate of infection was higher in October and lowest in January. The overall prevalence of infection during the study period was 46.8%. Infection was higher among the birds of age 3-8weeks (56.12 %) as compared to the 9-20 weeks age group (46.59%) and the >20 weeks age group (32.81%). A significant association (p=0.01) was observed between the age groups of chickens and the occurrence of disease. **Conclusions:** Current findings provide the baseline data of the disease in District Sialkot. The prevalence of *Eimeria* is very high and needs attention to develop control strategies before the onset of disease.

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 the effective control and 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. This study aimed 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 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 × p × (1-p)/d, 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. A total of 250 samples were taken from three different age groups (3- 8 weeks, 9-20 weeks, and >20 weeks). of hens and cocks/ during a period from August 2019 to January 2020. These groupings were based on commonly recognized poultry production stages, representing early growth, grower phase, and mature birds, respectively. These intervals 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, the average temperature ranged between 35°C and 15°C. The average humidity during the sampling period ranged between 59% and 40%. Samples were initially diluted and then strained to remove the debris. After the strained sample was placed on the bench top and left for 10-15 minutes for the sedimentation of oocysts. Then, the samples were examined under a microscope to detect the presence of oocysts in feces. From the positive samples, non-sporulated oocysts were isolated by using the saturated saline flotation and centrifugation method, as shown in figure 1.

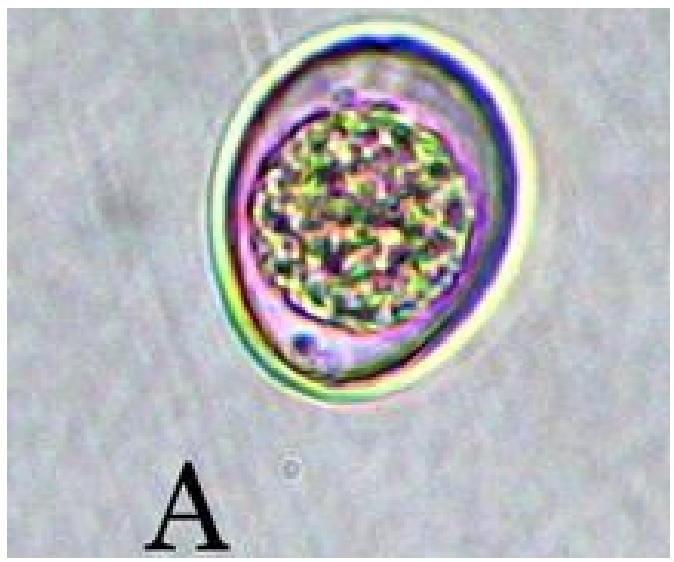

Figure 1: Nonsporulated Oocyst

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 for further identification, as shown in figure 2.

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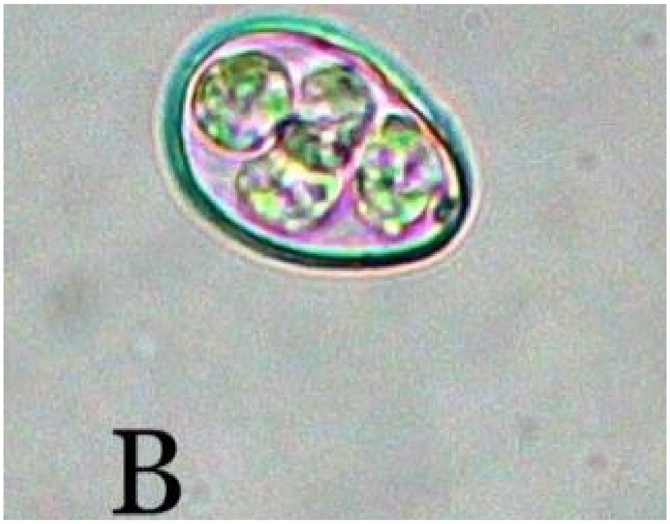

Figure 2: Sporulated Oocyst

Sporulated occysts were examined under 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. *Eimeria* were identified by following the guidelines given by Mares *et al.* [11].

The data were analyzed to determine the percentage prevalence. It was calculated by dividing No. of positive samples by total No. of samples and multiplying by 100. 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 disease at a confidence interval of 95%. 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 found as 46.8% with 95% confidence interval 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 (%)	95% Confidence Interval
August, 2019	40	18 (45%)	29.7%- 60.2%
September, 2019	55	29 (52.72%)	39.5%-65.9%
October, 2019	60	36 (60%)	47.6%-72.4%
November, 2019	50	21 (42%)	28.2%-55.6%
December, 2019	25	09 (36%)	17.1%- 54.8%
January, 2020	20	04 (20%)	2.47%- 37.5%
Total	250	117 (46.8%)	40.6%- 52.9%

The prevalence of infection was highest (56.12 %, 55/98) with 95% confidence Interval of 46.3-61.0% 3-8weeks aged chickens. In the case of the 9–20-week age group, prevalence was 46.6% (41/88; 95% CI: 36.1-57%). The lowest rate of disease, 32.81% (21/64; 95% CI: 21.3-44.3%), was found in the>20 weeks and above age group. Prevalence of disease in each age group is shown in figure 3.

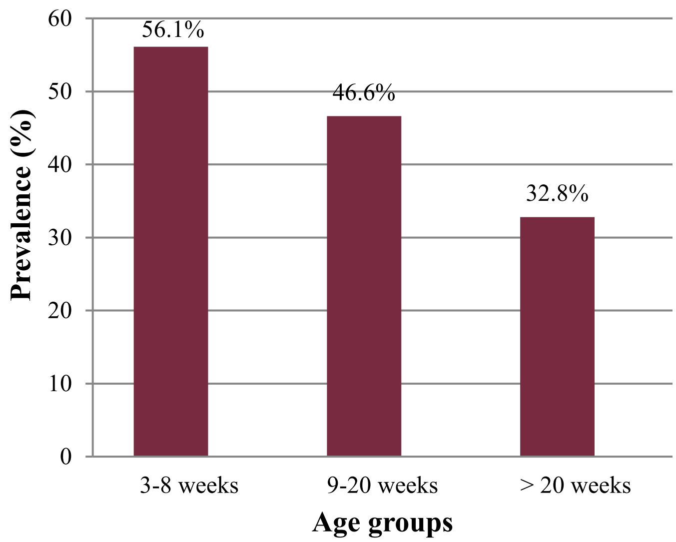

Figure 3: Overall Percentage Prevalence of Coccidiosis in Each Age Group of Chickens

A total of 123 samples were taken from cocks. Out of these, 53 (43.08%; 95% CI: 34.3-51.8%) samples were recorded as positive. In case of hens, 64 (50.39%; 95 CI: 41.7-59%) were positive out of 127 samples. The rate of coccidiosis was higher in females than in males, as shown in figure 4.

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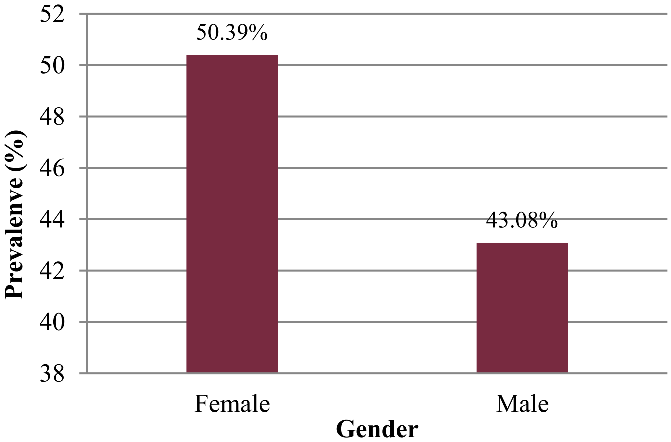

Figure 4: Overall Percentage Prevalence in Females and Males

Results of Chi square revealed that there was a non-significant relation between gender and prevalence of disease. Significant variation was observed in different age groups' infection (p=0.01), while monthly prevalence also showed a significant difference (p=0.03). The results of the association of different factors with coccidiosis are given in table 2.

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

Risk Factors	Total Samples	Positive Cases	Prevalence (%)	X^2	p-value
Gender					
Male	123	53	43.08	1.33	1.24
Female	127	64	50.39		
Age Group					
3-8 Weeks	98	55	56.12	8.45	0.01
9-20 Weeks	88	41	46.59		
9-20 Weeks	64	21	32.81		
Months (Aug-Jan)	250	117	46.8	12.43	0.03

On the basis of morphological characters, five species of *Eimeria* were identified from chickens in Sialkot. Measurements of length, width, and shape index of sporulated oocysts (in micrometers) and identified species are given in table 3.

Table 3: Measurements of Oocysts and Identified Species

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

Five different species were identified in *Eimeria*, like (A) *E. maxima*, (B) *E. acervulina*, (C) *E. mitis*, (D) *E. tenella*, and (E) *E. necatrix*, as shown in figure 5.

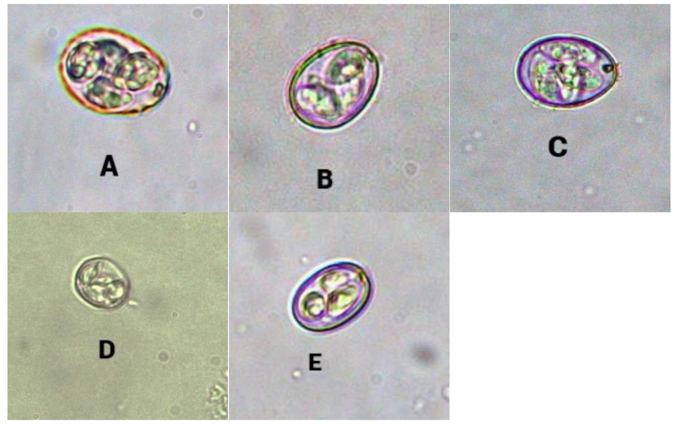

Figure 5: Identified species of Eimeria: A: E. maxima, B: E. acervulina, C: E. mitis, D: E. tenella, E: E. necatrix

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 the 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 Azam *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. Andreopoulou *et al.* accounted 9.59 % prevalence in Mirpur (Azad Kashmir) [5]. Amin *et al.* reported a percentage 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]. Khursheed reported 58% prevalence in Southern Punjab [15]. In the District of Swabi (Pakistan), Begum *et al.* documented 67.57% of disease [4]. 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 for the high cases in these months may be because of favorable temperatures 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

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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 the months 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, 16]. 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 3-8 weeks age group, followed by 9-20 weeks old chicks, while the lowest prevalence was found in the>20weeks age group birds. These results are in harmony with findings of Bachaya *et al.* and Naqvi *et al.* who reported that the infection was more common in younger chickens in comparison to adults [2, 17]. A feasible reason for the higher rate of prevalence of coccidian infection in the 3–8-week 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 group. 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 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 females (20.1%) as compared to males (18.5%) [18]. Contrary to current findings, Alemayehu *et al.* observed a higher frequency of disease in males than females in Addis Ababa, Ethiopia [19]. In the current study, five species of *Eimeria* were identified on the basis of the morphology of oocysts. Among the identified species, oocysts of *E.mitis* were of the smallest size and sub-spherical in shape. Oocysts of *E. maxima*, tenella, and acervulina were oval in shape. *E. necatrix* were oblong in shape. Oocysts of *E. maxima* were the largest and showed a specific 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 responsible for intestinal coccidiosis, *E. necatrix* and *E. maxima*, are also highly pathogenic, while *E. mitis* and *E. acervulina* have low pathogenicity [20]. Findings of Sohail *et al.* were partially in harmony with the 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 [16].

This was a cross-sectional study in District Sialkot, which employed convenience sampling of the available farms and therefore could not be generalized to all poultry operations in the area. Species identification was based only on microscopic morphology without molecular confirmation (PCR), and there was a risk of misclassifying closely related species. The six-month study period (August to January) failed to capture the dynamics of transmission during spring and early summer, and the absence of data on farm management, anticoccidial use or vaccination status did not measure important confounders. Practical evidence could be produced through intervention research testing region-specific control strategies: 3–8-week flocks of warm months should be prophylactically targeted using improved litter management and rotational use of anticoccidial control, using randomized controlled trials. Economic impact analyses that would match the count of oocysts on the farm with losses in production (weight gain, mortality, feed conversion) would be a good justification for the farmer to take preventive steps.

CONCLUSIONS

The results showed that coccidiosis is moderately prevalent in District Sialkot. Both forms of disease, intestinal and caecal coccidiosis confirmed to be present in Sialkot poultry. 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: AWQ
Formal analysis: 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.

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Conflicts of Interest

All the authors declare no conflict of interest.

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