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Prevalence and Risk Factors of *Avian Coccidiosis* in Domestic Chickens *(Gallus Gallus Domesticus)* In Tehsil Matta, Swat, Pakistan

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Abstract

Background: *Avian Coccidiosis* is an important parasitic disease of poultry production worldwide caused by *Eimeria* spp. In Pakistan, in rural areas where veterinary services are limited, its prevalence and impacts are not well documented. **Objective:** To determine the prevalence, risk factors and clinical effects of coccidiosis in domesticated chickens (*Gallus Gallus Domesticus*) in Tehsil Matta, Swat, Pakistan. **Methods:** A cross-sectional study was carried out from March to July 2025. A total of 750 chickens underwent clinical examination for signs of coccidiosis. *Eimeria* oocysts were detected in fecal samples from suspected cases (90) by the flotation method. Structured questionnaires were used to gather data on potential risk factors such as age, gender, housing Conditions, season and vaccination status, which were subsequently analyzed. **Results:** The overall prevalence of coccidiosis was 8.0% (60/750). The infection rate was higher in female chickens (75%, n=45) than in males (25%, n=15). The most susceptible age group of young chickens (up to 3 months old) accounted for 65% (n=39). Key risk factors that were significantly associated with infection were crowded housing (80%, n=48), summer season (88.3%, n=53), and not being vaccinated (91.7%, n=55). The high rates of infection were also associated with poor sanitation. Clinical effects consisted of decreased feed consumption, diarrhea, ruffled feathers and a 30% (18/60) mortality rate in infected birds. **Conclusion:** *Avian Coccidiosis* is common in domestic chickens in Tehsil Matta and the young, unvaccinated chickens are most susceptible in overcrowded and unsanitary Conditions. The disease results in substantial mortality and reduced productivity and there is a need for better management, biosecurity and awareness programs in the region.

Introduction

Poultry comprises all domesticated birds including chickens, turkeys, ducks, and geese, which are primarily raised for meat and egg production. Among these, chickens are the most common poultry species, having adapted to diverse climates and environments and significantly contributing to human nutrition through animal derived proteins [15]. In many developing countries, poultry plays an important role in agriculture and household economies, often serving as a pathway out of poverty for landless rural families [4]. Despite the importance of poultry, several problems affect poultry production systems, such as diseases, deficiency of nutrients, predation, and weak management practices etc. *Coccidiosis* is known as one of the important parasitic diseases of poultry and is caused by protozoan parasites of the genus *Eimeria*. The disease seriously impacts the growth and feed utilization of infected birds resulting in decreased productivity. It is one of the serious poultry parasitic diseases that affect the epithelial lining of the bird's intestine worldwide [17]. Most *Eimeria* species affect birds, between 3 and 18 weeks of ages and can cause high mortality in young chickens [37].

Morphologically, *Eimeria* oocysts are ellipsoidal or spherical shape with thick walls and sporocysts. *Eimeria* maxima is the largest (30×20µm), whereas *Eimeria* mivati (17×15µm) and *Eimeria* mitis (16×15µm) are among the smallest. Most species are ovoid in shape, except *Eimeria* necatrix, which is oblong [16]. *Eimeria* parasites are protected by a glycoprotein and lipid bilayer structure known as the oocysts wall, which encapsulates their soft bodies [34]. This wall allows oocysts to survive in harsh environmental Conditions for extensive periods [20].

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Eimeria oocysts have a hard, multilayered outer wall, which makes them quite resistant to most sanitizers but can be destroyed by high temperature (above 50°C) or exposure to ammonia [25]. The concentration of humidity in the environment also plays an important role such as water spills or heavy rainfall create high humidity (over 60%), which help *Eimeria* oocysts survive [29,38,6]. In many tropical and sub-tropical region, chickens are raised in open housing system, especially in backward setups. [23]. In this outdoor environment, when Conditions are ideal i.e. temperature between 25 to 30°C, about 75% humidity and good air movement then sporulated *Eimeria* oocysts can survive for up to 602 days [19].

Nine Eimeria species are identified as causative agents of poultry *coccidiosis*, but only seven of them have been reported to be pathogenic [28]. *Eimeria* necatrix and *Eimeria* tenella are the most pathogenic, *Eimeria* acervulina, Eimeria maxima, and *Eimeria* mivati are common and slightly to moderately pathogenic, while *Eimeria* brunti is uncommon but pathogenic when it does occur, *Eimeria* mitis, *Eimeria* praecox and *Eimeria* hagani are relatively nonpathogenic species [44]. These parasites are highly host specific and primarily develop in in the epithelial cells of the chicken's intestinal villi or crypts [46].

Clinical signs include poor weight gain, reduced feed efficiency, and slower growth rates, all of which contribute significantly to economic losses [45]. Major risk factors include poor litter management, especially failure to remove wet litter. Other contributing factors include environmental and physiological stress, such as rapid dietary changes, concurrent infections, and excessive use of coccidiostats (chemical compounds). [10]. Infections are most common in chickens aged 3 to 18 weeks, with younger chicks being at greater risk of mortality [3].

To detect *Eimeria* oocysts in feces a special solution (flotation solution) with higher specific gravity (i.e. saturated NaCl + sugar) or concentrated zinc sulphate solution is used that allow the lighter oocysts to float to the surface and sink other debris to the bottom of solution. This helps to isolate the oocysts for viewing [39]. Another method is carried out to determine the number of oocysts per gram of feces (OPG count).it counts how many oocysts are in one gram of feces. The McMaster method is used, which involves a special slide to count oocysts under microscope. It also determines how many oocysts are mature and measures their size. [17].

In developing country like Pakistan majority of the poultry farm is in backyard and veterinary services are not readily available which is one of the major challenges in poultry production in Pakistan [6, 29]. In rural areas like Tehsil Matta, Swat, there is little baseline information on the prevalence and effects of the *coccidiosis*, especially in areas where level of modern poultry keeping is low. A thorough knowledge of the local epidemiology of the disease is essential to be able to create effective and sustainable control measures. With this in view, the present study has been carried out with the following specific objectives:

- To find out the prevalence of *Avian Coccidiosis* in domesticated chicken (*Gallus Gallus Domesticus*) in Tehsil Matta, Swat.
- To determine the risk factors (such as age, gender, housing, season, vaccination status and sanitation) associated with the infection.
- To evaluate clinical symptoms and clinical changes of the disease in affected birds.
- To assess the economic losses due to mortalities, weight loss and decreased egg production in the study area caused by *coccidiosis*.

The results of this research will be used to supply scientific information and recommendations to address *Avian Coccidiosis* in resource-limited poultry production system.

Materials and Methods

❖ **Study Area**

The study was conducted in various areas of Tehsil Matta, District Swat in Khyber Pakhtunkhwa Province of Pakistan. The geographical features of Tehsil Matta are a hilly area of land in between 34°55' to 35°10' North latitude and 72°15' to 72°30' East longitude and has an average height of 1200 meters above sea level. The climate is subtropical and hot in summers (25-40°C) and cold in winters with humidity ranging from dry to humid.

❖ **Study Design and Sampling Strategy**

The study was designed as a descriptive survey from March to July, 2025, with the aim of assessing the prevalence, risk factors and clinical effects of *Avian Coccidiosis* in domestic chickens of Tehsil Matta, Swat.

❖ **Sample Size Calculation**

The required sample size was determined by the formula for cross-sectional studies:

text $n = (Z^2 \times P \times (1-P)) / d^2$

Where: Z = 1.96 (95% confidence level)

P = 50% (0.5) - expected prevalence (used as no previous data was available for the study area)

d = 5% (0.05)

Desired precision text $n = (1.96^2 \times 0.5 \times 0.5) / 0.05^2$ n = 384.16 ≈ 385

The sample size was expanded to 750 chickens in the event that some chickens might not respond.

❖ **Sampling Strategy**

A multi-stage sampling technique was used:

- **Stage 1 (Selection of Villages):** Tehsil Matta was segmented into four geographical areas (North, South, East and West). 8 villages were selected at random in each zone (2 villages/zone).

- **Stage 2 (Selection of Households/Farms):** A total of 10-15 households/farms with domesticated chickens were randomly selected from each village.
- **Stage 3 (Selection of Chickens):** All chickens were included from selected households/farms.

❖ **Inclusion Criteria**

- All domestic fowl (*Gallus Gallus Domesticus*) of all ages, sexes, and breeds.
- Domesticated chickens that are raised in backyard or semi-intensive Conditions.

❖ **Exclusion Criteria**

- Chickens treated with anticoccidial drugs within 7 days before sample collection.
- Severely debilitated, that is, moribund chickens with other diseases.
- Commercial intensive systems are the type of houses where chickens are kept.

Data Collection

❖ **Questionnaire Survey**

A pre-tested and structured questionnaire was used for face-to-face interviews with the chicken owners, to gather data on demographic information, management practices and clinical signs. The following data was captured:

- An overview of chicken information involving age, gender and breed
- Management Practices: Housing type, crowding density, sanitation Conditions.
- Health History: Immunization status, past disease outbreaks.
- Pathogenicity: Diarrhea, decreased feed consumption, weight loss, ruffled feathers, death.
- Economic Data: Average weight of healthy birds vs. infected birds, egg production in layers.

❖ **Clinical Examination**

A clinical examination was performed on all 750 chickens to determine clinical symptoms potentially associated with *coccidiosis*, using a thorough physical examination. General Conditions of the body, Conditions of feathers, comb and wattle, consistency of feces and feed consumption were recorded.

Sample Collection

All chickens exhibiting clinical signs suggestive of *coccidiosis* (n=60) and a random sample of apparently healthy chickens (n=30) were sampled for laboratory confirmation of *coccidiosis*. Samples were collected from the cloaca using sterile gloves or taken after defecation by using wooden sticks. About 5-10 grams of feces were obtained from each bird and transferred to a labelled sterile, leak-proof container. The collected sample was kept in an ice pack and transferred to the Laboratory of the Department of Zoology, Government Afzal Khan Lala Post Graduate College, Matta, Swat within 4-6 hours of collection.

❖ **Rationale for Sampling Strategy**

Fecal flotation is a time-consuming process. Performing fecal flotation on all 750 chickens was not logistically possible nor is it scientifically necessary. Instead:

- All clinically suspected cases (n=60) were tested to confirm the diagnosis.
- To estimate the subclinical infection rate apparently healthy chickens (n=30) were tested randomly.

This is commonly used in the majority of epidemiological studies and allows the efficient use of laboratory resources.

Laboratory Diagnosis

❖ **Fecal Flotation Method**

Fecal flotation method was used to identify *Eimeria* oocysts [17].

❖ **Reagents and Materials**

Preparation of saturated sugar flotation solution (S.G. =1.30) is done by dissolving 454g of sucrose in 355ml of distilled water. The test tubes, Glass slides, cover slips, wooden sticks, micropipette, centrifuge, light microscope are needed for the experiment.

❖ **Procedure**

Sample Preparation: About 3 g of freshly collected fecal sample was put into a test tube and homogenized with a wooden stick in 20ml of distilled water.

Filtration: The fecal fluid was passed through a double layer cheesecloth to remove big debris.

Centrifugation: The filtrate was centrifuged at 1500 rpm for 3 minutes. The sediment was kept and the supernatant discarded.

Flotation: The sediment was mixed with 5 mL of saturated sugar flotation solution and thoroughly mixed.

Final Centrifugation: The mixture was then centrifuged again for 3 minutes at 1500 rpm.

Slide Preparation: Using a micropipette, 15µL of the supernatant was carefully collected from the surface and placed on a glass slide. A cover slip was placed over the sample.

Microscopic Examination: The slide was then examined with a light microscope at 10x and 40x magnification. *Eimeria* oocysts were identified by their characteristic morphology—

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shape, size, color and wall structure—following Conway and McKenzie [17].

Confirmation of *Eimeria* Oocysts: To confirm, the following characteristics were observed: Oocysts shape and size: Oocysts vary in shape from spherical, to oval and ellipsoidal and are 15-30 µm long and 13-24 µm wide [44]. The oocyst has a smooth wall that may be thick or thin.

Sporulation: When sporulated oocysts containing four sporocysts, containing two sporozoites [27], are present.

Ethical Considerations

- **Ethical Approval**

Prior approval for the study was granted by the Institutional Research Ethics Committee (IREC) of the Department of Zoology, Government Afzal Khan Lala Post Graduate College Matta, Swat (Approval No: ZOOL/IREC/2025/07).

- **Informed Consent**

All the chicken owners/farmers gave their prior informed written consent for the beginning of the study. The study was presented to the participants in their local language (Pashto) and procedures and potential risks were explained. Confidentiality of the data and the right to withdraw from the study was assured to the participants.

- **Animal Welfare**

All procedures involving chickens were conducted according to the OIE guidelines and the animal welfare act. Care was taken to reduce the stress and discomfort associated with clinical examination and sampling.

Statistical Analysis

Data were entered into the Microsoft Excel 2019 and analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

- **Descriptive Statistics**

The prevalence was defined as: text Prevalence (%) = (Positive chickens / Total number of chickens examined) × 100

- **Inferential Statistics**

Categorical risk factors (gender, age group, vaccination status, season, crowding, sanitation) were tested for association with *coccidiosis* infection by the Chi-square test (χ^2). To measure the association between risk factors and infection 95% Confidence Intervals (CI) were computed. A P-value of < 0.05 was considered statistically significant.

Results

The primary objective of this study was to assess the prevalence of *coccidiosis*, identify associated risk factors, and evaluate its overall impact on domesticated chickens in Tehsil Matta, District Swat.

- **Overall Prevalence**

A total of 60 (8.0%) chickens were diagnosed as positive for *coccidiosis*, based on clinical signs and confirmed by fecal flotation, out of 750 chickens examined. Prevalence for the overall data is presented in Table 3.1 with a 95% confidence interval.

Table 3.1: Overall prevalence of *Avian Coccidiosis* was 8.0% (60/750; 95% CI: 6.2–10.1).

Parameter	Number Examined	Positive	Prevalence (%)	95% CI
Total Chicken	750	60	8 %	6.2-10.1

- **Gender Wise Prevalence of *Coccidiosis* Disease**

A higher infection rate was seen in females (75% 45/60) than in males (25% 15/60). There was a very significant association between gender and infection ($\chi^2 = 38.45$, df = 1, $P < 0.001$). Of the total number of chickens observed, a higher number of females (700/750; 93.3%) were observed as compared to males (50/750; 6.7%) which contributed to the higher number of infected females. The prevalence of *Coccidiosis* by gender is presented in table 3.2.

Table 3.2: The gender wise prevalence of *coccidiosis* disease

Gender	Examined	Infected	Prevalence (%)	X^2	df	P-value
Male	50	15	30%			
Female	700	45	6.4%	38.45	1	<0.001
Total	750	60	8.0			

- **Age-Wise Prevalence of *Coccidiosis* Diseases in Male Chickens**

Among male chickens, young (≤3 months) showed the highest prevalence (66.7%; 10/15), followed by adults (20.0%; 3/15) and middle-aged (13.3%; 2/15) (Table 3.3).

Table 3.3: The prevalence of *coccidiosis* in male chickens by age group.

Age Group	Examined	Infected	Prevalence (%)
Young (up to 3 months)	33	10	30.3
Median (from 3 to 4 months)	8	02	25.0
Adult (above 4 months)	9	03	33.3

❖ Age Wise Prevalence of *Coccidiosis* Diseases in Female Chickens

Among female chickens, young birds (≤3 months) showed the highest prevalence (64.4%; 29/45), followed by adults (22.2%; 10/45) and middle-aged birds (13.3%; 6/45) (Table 3.4).

Table 3.4: The age-wise prevalence of *coccidiosis* in female chickens

Age Group	Examined	Infected	Prevalence (%)
Young (up to 3 months)	330	29	8.8
Median (from 3 to 4 months)	205	6	2.9
Adult (above 4 months)	165	10	6.1

❖ Combined Age Wise Prevalence

Overall, young chickens (≤3 months) showed the highest prevalence (65.0%; 39/60), followed by adult chickens (21.7%; 13/60) and middle-aged chickens (13.3%; 8/60). There was a significant difference in prevalence by age group ($\chi^2 = 10.23$, df = 2, P = 0.006) (Table 3.5).

Table 3.5: Combined Age-Wise Prevalence of *Coccidiosis*

Age Group	Examined	Infected	Prevalence (%)	X^2	Df	P-value
Young (up to 3 months)	363	39	10.7			
Median (from 3 to 4 months)	213	08	3.8			
Adult (above 4 months)	174	13	7.5	10.23	02	0.006

❖ Risks Factors of *Coccidiosis* Diseases

Area of Living

80.0% (48/60) of chickens reared in more crowded area showed significantly higher infection rates than less crowded area (20.0% (12/60)). There was a strong relationship between crowding and infection ($\chi^2 = 21.60$, df = 1, P < 0.001) (Table 3.6).

Table 3.6: The association between *Coccidiosis* and Crowding

Crowding	Examined	Infected	Prevalence (%)	X^2	Df	P-value
Less Crowded	350	12	3.4			
More Crowded	400	48	12	21.60	1	<0.001
Total	**750**	**60**	**8.0**			

Seasons

88.3% (53/60) of the cases were found in the summer season and 11.7% (7/60) in the spring season. The association between season and infection was very significant ($\chi^2 = 35.27$, df = 1, P < 0.001) (Table 3.7).

Table 3.7: Association between Season and *Coccidiosis*

Season	Examined	Infected	Prevalence (%)	X^2	Df	P-value
Spring (Dry)	350	07	2.0			
Summer (Warm and Humid)	400	53	13.3	35.27	1	<0.001
Total	750	60	8.0			

Vaccination

The prevalence of unvaccinated chickens (91.7%; 55/60) was significantly higher than for vaccinated chickens (8.3%; 5/60). The relationship between vaccination status and infection was highly significant ($\chi^2 = 41.67$, df =1, P<0.001) as seen in Table 3.8.

Table 3.8: No association between the vaccination and the *coccidiosis*

Vaccination	Examined	Infected	Prevalence (%)	X^2	Df	P-value
Vaccinated	350	05	1.4			
Non-Vaccinated	400	55	13.8	41.67	1	<0.001
Total	750	60	8.0			

The prevalence among chickens kept under poor sanitary conditions was significantly higher (95.0%; 57/60) than the good sanitation Conditions (5.0%; 3/60). The relationship between sanitation and infection was significantly high (Table 3.9), ($\chi^2 = 48.60$, df = 1, P < 0.001).

Table 3.9: No association between sanitation and *coccidiosis*

Sanitation	Examined	Infected	Prevalence (%)	X^2	Df	P-value
Good Sanitation	350	03	0.9			

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Poor Sanitation	400	57	14.3	48.60	1	<0.001
Total	750	60	8.0			

❖ Summary of Risk Factors

All risk factors and their statistical significance are summarized in Table 3.10.

Table 3.10: A summary of the risk factors for *coccidiosis*

Risk Factors	Category	Prevalence (%)	X^2	Df	P-value
Gender	Male	30.0	38.45	1	<0.001
	Female	6.4			
Age	Young	10.7	10.23	2	0.006
	Middle	3.8			
	Adult	7.5			
Crowding	Less Crowded	3.4	21.60	1	<0.001
	More Crowded	12.0			
Season	Spring	2.0	35.27	1	<0.001
	Summer	13.3			
Vaccination	Vaccinated	1.4	41.67	1	<0.001
	Unvaccinated	13.8			
Sanitation	Good	0.9	48.60	1	<0.001
	Poor	14.3			

❖ Impacts of *Coccidiosis* on Economy

Meat Quality and Quantity

Table 3.11 shows the effect of *coccidiosis* on meat production. Significant weight loss was observed in the infected chickens (50% in young and 75% in adults) as compared to the healthy chickens.

Table 3.11. Meat Production affected by *coccidiosis*

Status	Age Group	Frequency	Average meat in kg	Weight Loss (%)
Healthy chickens	Young (up to 3 months)	363	1kg	-
	Middle (3 to 4 months)	213	1.5kg	-
	Adult (above 4 months)	174	2kg	-
Infected chickens	Young (up to 3 months)	39	0.5kg	50%
	Middle (3 to 4 months)	8	1kg	66%
	Adult (above 4 months)	13	1.5kg	75%

Reduction of Eggs in Case of Egg Laying Chickens

The effect of *coccidiosis* on egg production is shown in Table 3.12. There was a significant decrease in egg production (16.0%; 10/60) among infected laying hens compared with healthy hens.

Table 3.12: The effect of *coccidiosis* on egg production

Status	Eggs production	Number of Hens	Percentage
Healthy Hens	Normal	165	100.0
Infected Hens	Decrease	10	16.0

Clinical Signs Associated with *Avian Coccidiosis*

The clinical signs observed in infected chickens are presented in Table 3.11. The most common clinical sign was watery diarrhea (95.0%; 57/60), followed by ruffled feathers (88.3%; 53/60), reduced feed intake (80.0%; 48/60), and weight loss (75.0%; 45/60). Only 3 (5.0%) of the affected adult chickens had bloody diarrhea. The mortality rate was 30.0% (18/60) in infected chickens.

Table 3.13: The clinical signs of infected chickens

Clinical signs	Number of affected	Percentage
Watery Diarrhea	57	95.0
Ruffled Featehers	53	88.3
Reduced Feed Intake	48	80.0
Weight Loss	45	75.0
Lethargy/Depression	42	70.0
Bloody Diarrhea	03	5.0
Mortality	18	30.0

Results of Floatation Method for *Avian Coccidiosis*

Ninety fecal samples were collected and examined using the fecal flotation method. Of the 60 samples which were positive for *Eimeria* oocysts, 30 samples (33.3%) were negative (Table 3.14).

Table 3.14: The results obtained from the fecal flotation method

Parameters	Number	Percentage
Total no of fecal samples	90	100.0
Positive	60	66.66
Negative	30	33.33

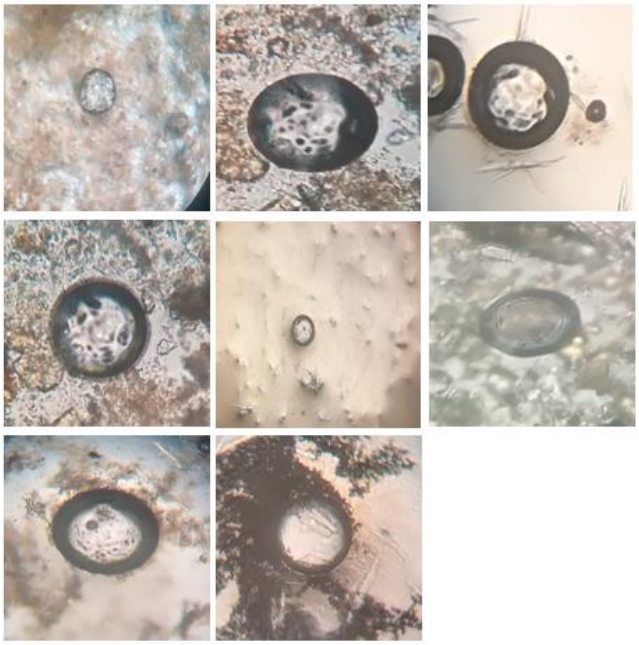

Figure 3.1 *Eimeria* oocysts were isolated from the feces of domestic chickens in the village of Matta in Tehsil Matta, Swat and the photomicrographs were taken at 400x magnification. Ovoid shape oocysts with smooth wall (A). (B) Ellipsoidal oocysts with typical shape. (4) Four sporocysts in a sporulated oocysts. Scale bar = 10 µm.

Discussion

Coccidiosis is still one of the most important parasitic disease of poultry and causes significant economic losses in the poultry industry worldwide, especially in developing countries where backyard poultry farming is the norm and veterinary services are not available. The present study was undertaken to find the prevalence, risk factors, clinical effects and economic losses due to *coccidiosis* in domesticated chickens at Tehsil Matta of Swat, Pakistan. The overall prevalence of 8.0% (60/750) found in this study provides baseline data for this area and highlights the need to improve the management practices.

The overall prevalence of 8.0% in this study is comparatively lower than some of the previous studies carried out in Pakistan, and other neighboring countries. In Pakistan, Awais et al. [6] reported high prevalence of 48.5% in broiler chickens of Faisalabad and Khan et al. (2006) reported 41.6% prevalence in Rawalpindi/Islamabad area. Likewise, Wondimu et al. [47] reported 42.2% prevalence in Gondar, Ethiopia and Debbou-louknane et al. [18] reported 56.7% prevalence in Algeria. There are a number of reasons for the low prevalence in our study. First, our study took place in a non-urban community with mainly small-scale backyard poultry keeping, whereas, many of the older studies were carried out on commercial broiler farms where *Eimeria* oocysts can be transmitted more readily at high densities of birds. Second, seasonal peaks may not be observed during the limited periods of time that the study took place (March through July 2025). Third, the sampling strategy used in our study only included clinically suspected birds rather than all birds for laboratory confirmation, which may have resulted in an underestimation of subclinical infections present in adult birds with partial immunity.

Several factors may account for the differences between the sexes: Often males have more physiological stress during growth than females which can affect immune function, making them more susceptible to infection with *Eimeria*. Furthermore, in many rural homes in the study area, male chickens are frequently kept in less space or given more freedom of movement, thereby exposing them to more contaminated environments. This is similar to results reported by Wondimu et al. [47] that found a higher prevalence of chicken pox infection (although not statistically significant) in male chickens.

The susceptibility of young chickens to infection with the *Eimeria* organism is probably greatest during the first few days of life, when the birds are have not yet developed immunity, from natural infection. This is a process that is documented extensively in the literature and is a characteristic of aging. Chickens aged between three and 18 weeks, and younger birds are most at risk of losing their lives from *coccidiosis*, according to Morris and Gasser [37]. Al-Natour et al. [3] also determined that chicks less than 8 weeks old were the most susceptible to clinical *coccidiosis*. Young birds have a high feed consumption and rapid growth rate, which allows *Eimeria* to multiply quickly in the intestinal tract. The difference observed in the prevalence between adult birds (21.7%) and our study may be explained by the fact that the birds had acquired immunity to *Eimeria* oocysts and by repeated exposure to these in the environment. Birds develop species-specific immunity and the infection is less severe as they get older, but they can still be reinfected. This phenomenon has been well described by Chapman et al. [14] and Blake and Tomley [7].

One of the strongest conclusions of this study is that there is a correlation between the housing density and the level of infections (80% of infected birds from crowded housing). Chickens are in closer contact with contaminated litter and feces, which are loaded with sporulated *Eimeria* oocysts that are infectious. The abundance of birds also leads to an abundance of oocysts in the environment, which makes it easier for birds to ingest. This result corroborates those of Graat et al. [24] who found that overcrowding was one of the most significant risk factors for *coccidiosis* in broiler flocks. Likewise, Kinung'hi et al. [31] found that overcrowding in both small and large-scale poultry farms was a significant risk factor for occurrence of *coccidiosis* in poultry farms in Ethiopia.

The higher prevalence in summer (88.3% of infected birds) than in spring (11.7%) is in line with the proven effect of environmental Conditions on *Eimeria* transmission. High temperatures (25-40°C) and high humidity (>60%) of summer season is conducive for oocysts sporulation and survival in Tehsil Matta. Biological mechanism of seasonal variation is well understood. The temperature range for optimal

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sporulation of *Eimeria* oocysts is 25-30°C and humidity levels greater than 60% [19, 23]. Such Conditions are generally present in the summer and early autumn in subtropical areas such as Swat. Furthermore, elevated temperatures lead to the increase in metabolic rate of chickens and may result in greater feed consumption which may lead to greater exposure to contaminated feed and water. Wet litter Conditions are another consequence of the rainy season and it also aids in the survival of oocysts and sporulation.

The finding of unvaccinated chickens had significantly higher infections (91.7% of infected birds) highlights the importance of vaccination as a prevention tool. While not widely available or cheap enough for backyard poultry farmers in the study area to afford, those birds that did receive the anticoccidial vaccine did have significantly lower incidences of infection. This is in line with much of the literature available which shows the effectiveness of anticoccidial vaccines. Live attenuated vaccines have been proven to reduce the severity of *coccidiosis* and improve production parameters by Chapman et al. [14] and Sharman et al. [42] respectively. For vaccination to be effective, however, it must be administered properly, and the right *Eimeria* species must be present in the field. Vaccination may be especially advantageous in backyard situations where multiple species are present.

Good hygiene plays a critical role in controlling *coccidiosis*. *Eimeria* oocysts can persist in contaminated litter, soil and feeds for months, are highly resistant to environmental degradation [20, 25]. There is a strong link between environmental hygiene and an increase in the rate of infection. In the study done by Wondimu et al. [47] poor management such as infrequent litter cleaning and cleaning at all were significantly correlated with higher prevalence of *coccidiosis*. Likewise, Mersha et al. [10] reported that poor hygiene in poultry farm was one of the most important risk factors associated with *coccidiosis* outbreak on farms.

The clinical signs noted during this study (watery diarrhea, ruffled feathers, decreased feed intake, weight loss and lethargy) are typical of *coccidiosis* and have been well documented in the literature [17, 45]. The presence of a high percentage of watery diarrhea (95%) and low percentage of bloody diarrhea (5%) suggest that the predominant *Eimeria* species that infect the study area may be those that primarily infect the upper intestine (*E. acervulina, E. maxima*) rather than the ceca (*E. tenella, E. necatrix*) which are more likely to cause hemorrhagic diarrhea. At the 30% mortality rate, the disease is greatly affecting the birds in the study population. This is similar to the mortality rate reported by Kinung'hi et al [31] in Ethiopia (13-14.5% mortality) but higher than the normal mortality rates of average well managed commercial flocks where anti-coccidial drugs and vaccines are routinely used.

In our study 8.0% prevalence of *coccidiosis* was recorded which is definitely low in comparison to other investigations conducted in Pakistan and other developing countries. Awais et al. [6] reported 48.5% prevalence in commercial broilers at Faisalabad and Khan et al. [29] reported 41.6% prevalence in the Rawalpindi/Islamabad area. The lower prevalence of our study may be due to the different study design (targeted sampling in our study versus random sampling in the other studies), different management systems (backyard versus commercial), and different time periods of study. The pattern of risk factors in our study is, however, generally similar to that observed in the literature. The high prevalence in young birds, poor sanitation and overcrowding, and seasonal variations in prevalence are consistent with the results of previous studies [6, 24, 47]. The uniformity of the results indicates that the biological and epidemiological principles of the transmission of *coccidiosis* are alike in various geographical regions and management.

Poor sanitation was the most significant risk factor identified, and could be substantially mitigated through improved sanitation, including regular cleaning, litter replacement and clean water provision. Reducing overcrowding by increasing available space per bird or decreasing bird density, would result in a reduction of environmental oocysts concentrations and bird stress. There is a need to increase access and affordability of vaccination programs, as vaccination rates were significantly lower than expected within the study area, which reflects a major opportunity for disease control. Furthermore, farmer education initiatives that emphasize poultry health management may help address some of the knowledge gaps contributing to many of the risk factors identified.

The present study has several limitations. First, *coccidiosis* prevalence may be highly seasonal and this short duration of the study may not have provided enough information to reflect this. Secondly, the study is limited geographically (to the single tehsil) and the results may not be generalizable to other areas. Finally, molecular confirmation was unavailable with only clinical signs and fecal flotation used for species identification (diagnostic limitations).

With the results and constraints revealed in this study, several future research directions are suggested. Longitudinal studies conducted over several seasons and years are needed in order to account for the temporal variation of prevalence. The molecular characterization of *Eimeria* species by PCR could yield accurate species identification and determination of the intensity of the infection. A cost-effectiveness analysis of alternative control strategies (sanitation, vaccination, anticoccidials) would aid in determining the most cost-effective option for resource-poor farmers.

Conclusion

Therefore, the prevalence of *Avian Coccidiosis* was observed in the domestic chicken population of Tehsil Matta, and young and unvaccinated birds in summer in the overcrowded, unhygienic animal market are at the highest risk of *Avian Coccidiosis*. The disease causes considerable clinical illness, mortality, and economic losses and thus there is a need to adopt better disease control and management strategies in the study area.

Recommendations

Based on the above conclusion the following recommendations are made:

- Poor sanitation habits were the most important risk factor with 95% of infections being caused by poor sanitation, so it is recommended to practice regular cleaning, dry litter maintenance and providing clean water.
- Minimize Stocking Density - to decrease stress and environmental oocysts burden.
- Anticoccidial vaccines should be made available and affordable; the unvaccinated birds had a much higher level of infection (91.7%).
- If temperature and humidity are conducive to oocysts sporulation, then additional preventive measures should be taken during this season.
- Training should be provided on disease identification, disease management and biosecurity.
- Enhance veterinary services in remote areas in order to provide timely diagnosis, treatment and advice.
- Longitudinal studies, molecular characterization of *Eimeria* species and evaluation of cost-effective control strategies are recommended.

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Authors' Contribution:

- All Authors Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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