



Diagnostic Approaches, Quantitative Assessment and Epidemiological Insights into Gastrointestinal Parasitism among Captive Peafowl (*Pavo cristatus*)

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ABSTRACT

Gastrointestinal parasitism is one of the most prevalent and common sanitary issue that can lead to sub clinical infection or even death. For detection and quantitative investigation, direct smear procedure, fecal flotation method and McMaster technique was employed. Coprological analysis of 90 fecal samples of *Pavo cristatus* (Indian peafowl), to identify and estimate the prevalence of gastrointestinal endoparasite infection, revealed highest prevalence (Mean $\pm$ SD) of 505 $\pm$ 81.31 at Murree Wildlife Park followed by 331.67 $\pm$ 162.67 at Jallo Wildlife Park and 216.67 $\pm$ 162.5 at Bahawalnagar Wildlife Park. The endoparasitic species recorded were *Strongyloides* sp., *Ascaridia* sp., *Trichuris* sp., *Ascaries* sp., *Heterakis* sp., *Eimeria* sp., and *Hymenolepis* sp. with site specific infection rates. Female birds showed more endoparasitic prevalence than males at all sites. Further evaluation of screening methods and diagnostic techniques, host-endoparasitic dynamics and integration of advanced therapeutics are prerequisite for curbing the subclinical infections of captive *Pavo cristatus*.

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SA: Research methodology, sampling, data analysis. SN: Conceptualization, reviewing and revising. AQ: Sampling and analysis. SS: Sampling and analysis. AR: Methodology, sampling and analysis. IAA, SB and RT: Revision and editing.

Key words

Pavo cristatus, Gastrointestinal parasites, Coprological, Prevalence, Sub-clinical, Therapeutics

INTRODUCTION

According to Anwar *et al.* (2015), the Indian peafowl (*Pavo cristatus*) is one of the many prominent and valuable avian species that are declining at an alarming rate in its natural habitats of Pakistan. *P. cristatus* are the only pheasant species, which are capable to adjust effortlessly in vicinities of humans and found in gardens, public parks and cultivated areas. As an omnivorous species, they feed on a wide range of food items, including grains, green crops, insects, and small reptiles. It can also be valuable

for the crops as bio-control by consuming harmful pests along with snakes and retain these venomous animals away from populated areas. Hence, the *P. cristatus* are dynamic ecological unit with effective services in an ecosystem. Its presence or absence influence the healthy quality of an ecosystem (Saim *et al.*, 2023).

Gastrointestinal infections are asymptomatic or symptomatic depending on the parasitic load (% prevalence). The symptoms of infected animal include weight loss, diarrhea and anemia especially in nestlings and juveniles and even cause death. Gastrointestinal endoparasitic infections indirectly reduce the host's immunity and distress the physical condition and can effect host survival and reproduction directly through such pathologies (Thawait and Maiti, 2015). The gastrointestinal endoparasites mainly effect the gastrointestinal tract of the bird and cause depression, anorexia, anemia, and death. Major endoparasites infections in *P. cristatus* are caused by *Coccidia*, gastrointestinal nematodes and cestodes (Jaiswal *et al.*, 2013). The worms reside the in the bird's intestine and lay cysts in the feces. Heavy infestations of

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these endoparasites distress the well-being of the birds gravely with reduction in weight of body, suppression of immune system against other diseases, retarded growth, low production of egg, emaciation and even death particularly in young birds (Mahnoor *et al.*, 2022).

There is a vast range of anthelmintic drugs available in the market that are effective against endoparasites. Although there is a large range of anthelmintic drugs available, endoparasites develop resistance to a particular drug when it is used repeatedly over time. Moreover, the chemical used in these anthelmintic does have side effects on cellular and molecular levels. In order to get best results from a drug, its properties should be suitable with internal body mechanisms of the bird. However, the control of endoparasites is primarily based on regular treatment with such anthelmintic (Esteban-Sánchez *et al.*, 2024). The current study was executed to determine the occurrence and diversity of endoparasites in *P. cristatus* at selected sites. Site and gender wise comparison was also computed to determine the differences among them.

MATERIALS AND METHODS

Site selection

Three selected Wildlife Parks for the current study had different management practices and different environmental conditions, which was helpful for the research study not only in a particular climatic zone (Köppen classification) but also in comparative analysis. Köppen climate classification system categorizes regions of the world into different climatic zones depending upon their vegetation (Sarfaraz *et al.*, 2014). The selected sites were Jallo wildlife park (climatic zone-BShw), Bahawalnagar wildlife park (climatic zone-BWhw) and Murree wildlife park (climatic zone-Cwb). *P. cristatus* was selected for the present study because of its presence at all selected sites, less than 10% of welfare studies carried out in zoos and aquariums over the past few years were focused on birds and the research on avian species is dominated by poultry than any other terrestrial bird species. All of the relevant information was documented on the data sheet prepared for on-site examination. During the fecal sample collection gender, color, density and presence of mucus and blood was observed and noted carefully. The total number of birds per cage, gender, feed, weight and age of selected birds was also noted.

Collection of fecal samples

Fecal cups were utilized to take fecal specimens. The cups were labelled with the sample number and date appropriately. The name of site and gender was also mentioned on the fecal cups. With the use of sterile

spatula, samples of feces (freshly egested) were collected in separate fecal cup (Otegbade and Morenikeji, 2014). From each selected site, 30 fecal samples were collected from *P. cristatus* comprising 90 samples collectively. To avoid cross contamination, sterile spatulas were used for each sample. Samples were collected carefully to avoid the contamination by sand or soil and the lids of fecal cups were closed immediately to prevent the loss of moisture from samples.

Fecal samples were kept in handling pack with ice packs (4°C). The fecal samples were stored in separate handling packs, along with ice packs to maintain a temperature of 4°C. All specimens were immediately transported to research facility for examination within 24 hours at the Zoology Department, Government College University in Faisalabad. Samples of feces could be kept at 4 °C for three days before analysis in case of delays.

Coprological analysis

Laboratory analysis of fecal samples was done by qualitative and quantitative techniques.

Qualitative analysis

Qualitative technique was used to indicate if the birds were harboring endoparasites and quantitative technique was used to estimate the sum total of eggs per gram (EPG) of endoparasites in fecal sample and their prevalence (%) in all examined birds. Qualitative examination of samples was done by using direct smear method and fecal floatation technique. Flagellated protozoan parasites can be detected by using the direct smear technique. However, compared to other methods, it determines prevalence at a lower rate. This technique is used to find trematodes and coccidian in bird fecal samples. A small quantity of fecal specimen was placed onto a clean glass slide, followed by the addition of a few drops of saline (NaCl) solution. The mixture was then thoroughly combined in equal proportions. The mixture was continuously stirred until a relatively homogenous texture was achieved. Large fecal fragments that were not properly mixed were taken off the slide. After covering the smear with a coverslip, the slide was examined under a microscope to identify the presence of endoparasites.

Fecal floatation technique is by far one of the most common diagnostic method to detect gastrointestinal parasites. It separates parasitic eggs, cysts and larvae based on differences in specific gravity (Soulsby, 1982). In a test tube, 2 g of fecal sample were combined with 10 ml of a floatation solution (sodium chloride solution). This mixture was then strained through cheesecloth, and the resulting filtrate was transferred to another test tube. The test tube was then filled by adding more floatation solution

and the test tube was covered with a cover slip on top and left to withstand for almost twenty min. After that, cover glass was removed and placed on a clear glass slide for examination under light microscope. Flotation with NaCl solution detects the majority of the infections by common helminths and non-sporulated coccidian oocysts/eggs at 10X and 40X magnification of microscope.

Quantitative analysis

In order to count eggs per gram (EPG) of feces, each fecal sample was analyzed by modified McMaster with the minimum detection limit of 50 eggs per gram. For this analysis, 2 g of fecal sample were taken in a suitable container. Then about 10ml of concentrated NaCl solution was added into it and gently mixed with the help of magnetic stirrer. This mixture was put through a strainer and filtrate was transferred into a glass test tube. The filtrate was left to stand for a duration of 15 min and then by utilizing a dropper, the McMaster Counting Chamber was filled carefully to avoid any bubble formation. The counting chamber was set and examined under light microscope for estimation of eggs/oocysts (Tanveer *et al.*, 2021). EPG were calculated for each sample by using following formula:

$$\text{EPG} = (\text{Chamber 1} + \text{Chamber 2}) \times 50$$

The occurrence of endo-parasites was estimated by using formula:

$$\text{Prevalence of parasites} = \frac{\text{no. of infected birds}}{\text{no. of examined birds}} \times 100$$

Statistical analysis

The outcomes of the laboratory examination were then analyzed statistically by version 21 of IBM's SPSS. A test for data normality Shapiro-Wilk and Levene's test was used to check the homoscedasticity of data

variance at significance of $P < 0.05$. The outcomes of the normality test were non-significant indicating the data was distributed normally (parametric data). To compute the significant differences ($P < 0.05$) between the means of endoparasitic species, one-way ANOVA was used. (Mean $\pm$ SD). Pair-wise comparison computed by Tukey's post hoc test between means of endoparasitic species EPG. Comparative analysis of endoparasitic means between birds of the selected sites was also done by ANOVA. Comparison between sites and gender for endoparasitic species was carried out to find out how they differ in endoparasitism infestation.

RESULTS

The details of gender-wise ratio of selected bird species at each site is given in Table I. Out of 90, helminthic infections were detected in 65 birds. The overall occurrence of endoparasites at Jallo Wildlife Park was 70%, Bahawalnagar wildlife park 63.33% and Murree wildlife park 83.33%. Comparative results of three sites showed that Murree Wildlife Park had highest number of infected birds (n=25) followed by Lahore (n=21) and Bahawalnagar (n=19) as presented in Table II. Out of the three selected sites, Murree wildlife park had highest EPG (47.94%) followed by Jallo wildlife park (31.49%) and Bahawalnagar wildlife park (20.57%) (Fig. 1).

The endoparasitic cysts identified from fecal samples of *P. cristatus* were as follow; one protozoan species *Eimeria* sp., six nematode species *Heterakis* sp., *Ascaridia* sp., *Ascaris* sp., *Trichuris* sp., *Strongyloides* sp., *Capillaria* sp., and one cestode species *Hymenolepis* sp. Site wise prevalence was recorded as, at Jallo wildlife park, 6 species of endoparasites were detected i.e., *Eimeria* sp.

Table I. Overview of the number of *P. cristatus* at each selected site.

Indian peafowl (<i>P. cristatus</i>)	Jallo wildlife park	Bahawalnagar wildlife park	Murree wildlife park
Number of female	136	30	22
Number of male	34	8	8
Total (with ratio of 4:1)	170	38	30

Table II. Prevalence (Mean $\pm$ SD) of endoparasites at Jallo Wildlife Park, Bahawalnagar Wildlife Park and Murree Wildlife Park.

Site	Total no. of birds	Total infected birds	Prevalence	EPG	Mean $\pm$ SD
Jallo wildlife park	30	21	70%	9950	331.67 $\pm$ 162.67 ^a
Wildlife park Bahawalnagar	30	19	63.33%	6500	216.67 $\pm$ 162.59 ^b
Wildlife park Murree	30	25	83.33%	15150	505 $\pm$ 81.31 ^c

Table III. Comparative endoparasitic species wise EPG (Mean $\pm$ SD) in Jallo Wildlife Park, Bahawalnagar Wildlife Park and Murree Wildlife Park.

Endoparasitic sp.	Test statistics	Jallo Wildlife Park	Bahawalnagar Wildlife Park	Murree Wildlife Park	Total Mean $\pm$ SD	P value
<i>Eimeria</i> sp.	Mean $\pm$ SD	225.00 $\pm$ 161.75 ^a	138.33 $\pm$ 131.10 ^b	290.00 $\pm$ 69.97 ^c	217.77 $\pm$ 140.09	0.00**
	EPG (%)	6750 (67.84%)	4150 (63.85%)	8700 (57.43%)		
<i>Ascaridia</i> sp.	Mean $\pm$ SD	23.33 $\pm$ 28.56 ^a	16.67 $\pm$ 37.90 ^a	71.67 $\pm$ 44.88 ^b	37.22 $\pm$ 44.70	0.00**
	EPG (%)	700 (7.04%)	550 (8.46%)	2150 (14.19%)		
<i>Strongyloides</i> sp.	Mean $\pm$ SD	48.33 $\pm$ 42.51 ^a	30.00 $\pm$ 38.51 ^a	30.00 $\pm$ 40.68 ^a	36.11 $\pm$ 41.07	0.14 ^{NS}
	EPG (%)	1450 (15%)	900 (13.85%)	900 (5.94%)		
<i>Ascaris</i> sp.	Mean $\pm$ SD	20.00 $\pm$ 40.68 ^a	8.33 $\pm$ 26.53 ^a	21.67 $\pm$ 50.32 ^a	16.67 $\pm$ 40.36	0.38 ^{NS}
	EPG (%)	600 (6%)	250 (3.85%)	650 (4%)		
<i>Heterakis</i> sp.	Mean $\pm$ SD	8.33 $\pm$ 23.06 ^a	6.67 $\pm$ 21.71 ^a	6.67 $\pm$ 21.70 ^a	7.22 $\pm$ 21.93	0.95 ^{NS}
	EPG (%)	250 (2.51%)	200 (3%)	200 (1%)		
<i>Hymenolepis</i> sp.	Mean $\pm$ SD	6.67 $\pm$ 25.37 ^a	5.00 $\pm$ 15.25 ^a		3.89 $\pm$ 17.13	0.30 ^{NS}
	EPG (%)	200 (2.01%)	150 (2.31%)	Nil		
<i>Trichuris</i> sp.	Mean $\pm$ SD	Nil	10.00 $\pm$ 20.34 ^a	Nil	3.33 $\pm$ 12.54	0.001**
	EPG (%)		300 (4.62%)			
<i>Capillaria</i> sp.	Mean $\pm$ SD			85.00 $\pm$ 72.10 ^a	28.33 $\pm$ 57.59	0.00**
	EPG (%)	Nil	Nil	2550 (16.83%)		

* P < 0.05 Significant; ** P < 0.01 highly significant. % = Prevalence of species at each site. NS, non-significant; D, standard deviation.

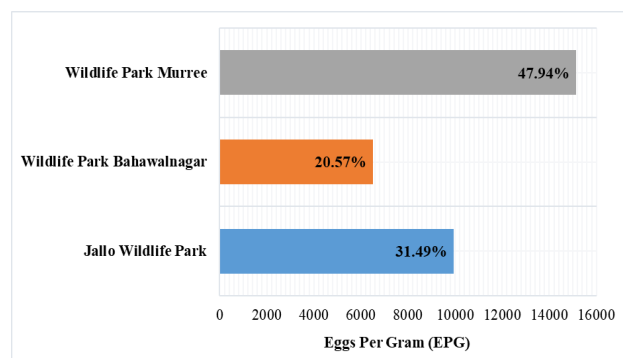


Fig. 1. Total EPG and prevalence (%) per site.

with highest prevalence followed by *Strongyloides* sp. > *Ascaridia* sp. > *Ascaris* sp. > *Heterakis* sp. > *Hymenolepis* sp. At Bahawalnagar Wildlife Park 7 endoparasitic species were found i.e., *Eimeria* sp. > *Strongyloides* sp. > *Ascaridia* sp. > *Trichuris* sp. > *Ascaris* sp. > *Heterakis* sp. > *Hymenolepis* sp. Murree Wildlife Park revealed prevalence of 6 endoparasitic species i.e., *Eimeria* sp. found highest followed by *Capillaria* sp. > *Ascaridia* sp. > *Strongyloides* sp. > *Ascaris* sp. Prevalence of *Eimeria* sp. was highest (217.77 $\pm$ 140.09) and lowest prevalence was recorded for *Trichuris* sp. (3.33 $\pm$ 12.54) among all detected species at all sites collectively. However, at each site,

endoparasitic species showed different rates of statistically significant (P<0.05) prevalence as shown in Table III. Overall, the site with highest endoparasitic prevalence was at Murree Wildlife Park while Bahawalnagar Wildlife Park showed lowest occurrence among selected sites.

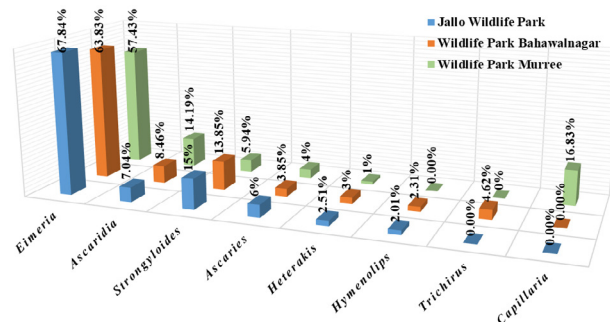


Fig. 2. Comparative prevalence (%) of endoparasites at each site.

Occurrence of *Eimeria* sp., *Ascaridia* sp., *Trichuris* sp. and *Capillaria* sp. indicated significantly different (P<0.05) prevalence rates of these endoparasitic species at each site, while *Strongyloides* sp., *Ascaris* sp., *Hymenolepis* sp. and *Heterakis* sp. had no significantly different (P > 0.05) occurrence rates between selected sites. At all

selected sites, the most prevailing endoparasitic species was *Eimeria* sp. showing highest prevalence of 67.84%, 63.85% and 57.43%. The comparative analysis of female and male infected birds showed different rates of infection among female and male birds, however the overall rate of endoparasitic species was found to be different for each endoparasitic species at all selected sites (Fig. 2).

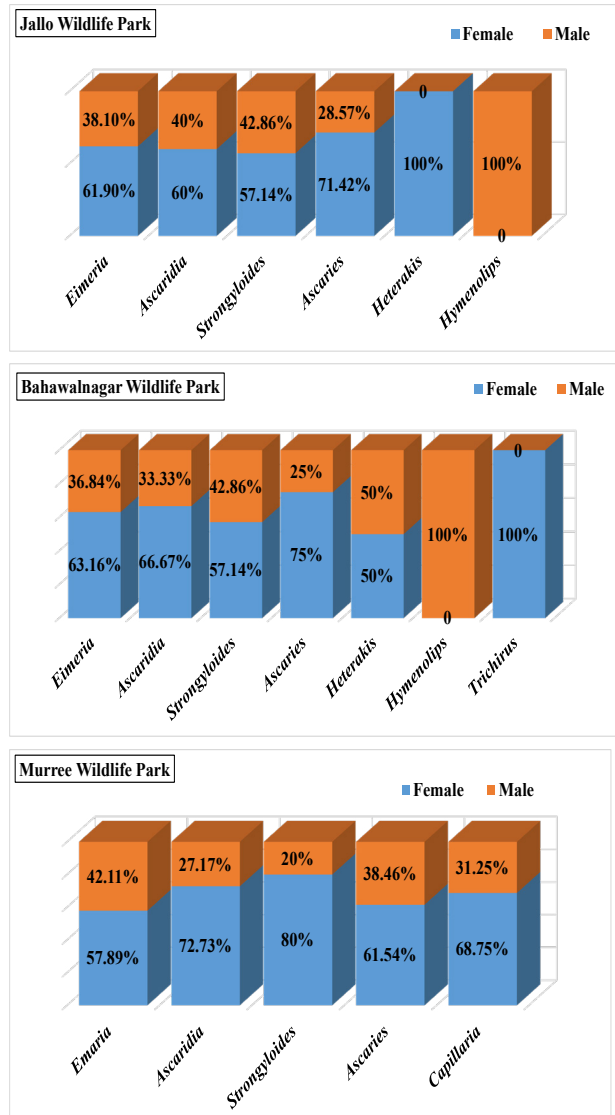


Fig. 3. Comparative analysis of endoparasitic species between female and male captive birds at three selected sites.

At Jallo Wildlife Park, among females, highest prevalent species recorded was *Ascaris* sp. (71.42%) and in the case of males, *Strongyloides* sp. (42.86%) was most prevalent, while lowest prevalent endoparasitic

species were *Strongyloides* sp. (57.14%) and *Ascaris* sp. (28.57%) in female and male captive birds, respectively. Moreover, *Heterakis* sp. was only recorded in females and *Hymenolepis* sp. only in males. In the case of Bahawalnagar Wildlife Park, *Ascaris* sp. (75%) was found highest in females and *Strongyloides* sp. (42.86%) in males with lowest prevalence of *Heterakis* sp. (50%) in females and *Ascaris* sp. (25%) in males. Another endoparasitic species, which was not detected in females, was *Trichuris* sp., *Strongyloides* sp. (80%) was found highest and *Eimeria* sp. (57.89%) was lowest in females, while *Eimeria* sp. (42.11%) was recorded highest and *Strongyloides* sp. (20%) was lowest in the case of males at Murree Wildlife Park. All the recorded species at Murree Wildlife Park were found in both females and males.

Site-wise comparison among selected sites showed that at Jallo wildlife park 13 females and 8 males were infected with prevalence of 61.90% and 38.10%, respectively. Similarly in Bahawalnagar wildlife park 6 males (31.58%) and 13 females (68.42%) were tested positive for endoparasitic infections. At Murree wildlife park, 16 females and 9 males with a prevalence of 64% and 36% were infected endoparasitic species. The results of infected females and males are presented for each sites in Figure 3. Highest prevalence of infestation was recorded in females (68.42%) at Bahawalnagar wildlife park and 31.58% infection rate in males at Jallo wildlife park among all analyzed captive birds. Overall, the rate of infection was higher in females with comparison to males at all sites (Fig. 4).

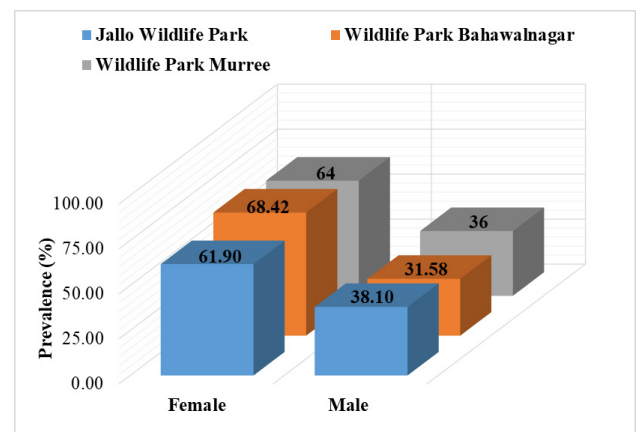


Fig. 4. Comparative analysis of number of infected female and male at Jallo wildlife park, Bahawalnagar wildlife park and Murree wildlife park

DISCUSSION

The present study was conducted at three selected

captive sites Punjab, Pakistan. Analysis of fecal samples of *P. cristatus* was conducted to investigate the occurrence of endoparasitic species and their prevalence from selected wildlife parks. Seven endoparasitic species were recorded in fecal samples of captive *P. cristatus* including *Eimeria* sp., *Strongyloides* sp., *Trichuris* sp., *Ascaridia* sp., *Heterakis* sp., *Ascaris* sp. and *Hymenolepis* sp. with different infection rate at each site.

These results from present study are in agreement with the work of Tanveer *et al.* (2021) who described prevalence of endoparasites at the same sites with comparable patterns. The study of Farooq *et al.* (2020) indicated 59.1% prevalence in Jallo Wildlife Park, Lahore and Basit *et al.* (2014) reported 58.3% prevalence in birds of prey and owls in Germany. Similar patterns were also recorded in the study by Akram *et al.* (2019), which involved a review for the incidence of gastrointestinal endoparasites in captive birds from several areas of Punjab, Pakistan. The 613 fecal samples were analyzed with an overall frequency of 54.32%. Another study by Setyowati *et al.* (2022) who reported 48-70% endoparasitic infestation in domestic chickens across an elevation gradient was also related to the present study.

The protozoan species i.e. *Eimeria* identified from present work revealed high prevalence at all sites as compared to the other endoparasitic species. This species mainly infect the upper small intestine and damage the mucosal cells leading to increased cell permeability, leakage of nutrients and plasma proteins, hindering the process of digestion and protein absorption. These effects contribute to both the clinical and subclinical manifestations of coccidiosis. Such disruptions severely disturbing the natural balance of gut, causing substantial malabsorption, decreased feed conversion, reduced weight gain, and an overall decline in productivity (Madlala *et al.*, 2021).

In the current investigation, *Eimeria* sp. emerged as the most prevalent parasite species across all three study sites 67.84%, 63.85 and 57.43% at Jallo Wildlife Park Lahore, Bahawalnagar wildlife park and Murree wildlife park, respectively. It aligns with the research study by Marniche *et al.* (2017). This study reported a higher prevalence of *Eimeria* sp. among blue peacocks in Ben Aknoun National Park (33.32 %) and El-Hamma test garden (58.31%) in Algeria. Yadav *et al.* (2021) reported coccidian prevalence in captive peafowls in Bangladesh. This study recorded 60% peafowls (30 out of 50 birds) infected with coccidia. The outcomes of present study also corroborate the research of Akram *et al.* (2019), who documented a prevalence rate of 67.87% for *Eimeria* sp. Similarly, Kathiravan *et al.* (2017) noted comparable results, with the highest incidence of *Eimeria* sp. (43.0%)

observed in freely roaming peafowls and much higher results were reported in captive *P. cristatus*) at São Paulo Zoological Park Foundation, Brazil with 91.7% coccidian oocysts (Rodrigues *et al.*, 2020).

Other reported species from this study consist of helminths comprising six nematode and one cestodes species. Among nematode species *Ascaridia* sp., *Strongyloides* sp., *Ascaris* sp. and *Heterakis* sp. were recorded at all three sites. *Strongyloides* sp. was most common at Jallo wildlife park (15%) and Bahawalnagar Wildlife Park (13.85%) with low occurrence at Murree Wildlife Park (5.94%). The results from Murree Wildlife Park closely related to the results of free ranging peafowls 4.17% (Kathiravan *et al.*, 2017). However, results from Bahawalnagar Wildlife Park (13.85%) are in accordance with Tanveer *et al.* (2021) with 20% *Strongyloides* occurrence while they reported much lower prevalence at Jallo Wildlife Park (3.3%) and higher at Murree Wildlife Park (16.67%) than present study. Marniche *et al.* (2017) found 16.70% *Strongyloides* in captive blue peafowl, which aligns with the results of present study from Jallo Wildlife Park. *Ascaridia* sp., *Heterakis* sp. and *Ascaris* sp. were reported at all selected sites with a range of 7.04-14.19%, 1-3% and 3.85-6%, respectively. The prevalence of *Ascaridia* sp. was in close accordance to the prevalence (6.9%) by Kathiravan *et al.* (2017) while Basit *et al.* (2014), Farooq *et al.* (2020) and Rodrigues *et al.* (2020) reported higher prevalence of *Ascaridia* sp. in peafowls as 26.53%, 33.33% and 63%, respectively that are contrary to the present work. The results of *Heterakis* sp. in accordance with El-Shahawy and Abou Elenien (2015) 3.3%. However, high prevalence was reported by other studies such as Farooq *et al.* (2020) 10% and Basit *et al.* (2014) 36.73% infection rate at Lahore Zoo. *Ascaris* sp. was also found at all study sites while Tanveer *et al.* (2021) who conducted a study at same sites reported this species only at Jallo Wildlife Park (6.67%).

Hymenolepis sp. was not found at Murree Wildlife Park, while other sites also had low prevalence rate as 2.01% and 2.31% at Jallo Wildlife Park and Bahawalnagar Wildlife Park, respectively which are contrary to the results of Kathiravan *et al.* (2017) where they detected *Hymenolepis* (4.16%) in the fecal samples of free-ranging Indian peafowl. Tanveer *et al.* (2021) had also documented occurrences of *Hymenolepis* sp. infection at Jallo wildlife park (10%) and Bahawalnagar Wildlife Park (13.33%). *Trichuris* sp. was only found at Bahawalnagar Wildlife Park (4.62%) in Indian peafowls, these results are in contradiction with the finding of Tanveer *et al.* (2021) who did not find this species at the same study site.

Opapa *et al.* (2010) quantified the prevalence of *Ascaris* sp., *Strongyloides* and *Trichuris* sp. cumulatively as 11.3%

in peacocks at Nekede zoological garden, which align with the present studies. *Capillaria* sp. with prevalence of 16.83% was only recorded at Murree Wildlife Park. A study conducted by Otegbade and Morenikeji (2014) reported similar prevalence of *Capillaria* sp. (16.2%) and Basit *et al.* (2014) 18.37% in peafowls while Farooq *et al.* (2020) quantified 26.67%, Ashraf *et al.* (2002) 59.62% and Rodrigues *et al.* (2020) 89.8% prevalence of *Capillaria* in common peafowl which is higher than current study.

Inclusively, the occurrence and diversity of endoparasitic oocysts fluctuated considerably at each site depending upon each captive bird's immunity and the physical condition. The prevalence of endoparasite species in the area under study can be attributed not only to their biological characteristics but also to various external factors. These factors encompass climatic conditions, environmental zones, infective eggs or larvae deposition in environment, availability of the secondary host, and the vulnerability of the definitive host. Temperature and moisture play pivotal roles as determinants influencing the incidence and extent of helminthiasis infections, affecting transmission through the persistence of parasites in environment and the success of their developmental stages (Sharma *et al.*, 2017).

The three selected sites exhibited distinct geographical and climatic characteristics. Setyowati *et al.* (2022) conducted a study on domestic chicken farms to find the prevalence and diversity of endoparasites within an elevation gradient. The results revealed that endoparasitic infestation was significantly higher at low elevation area (70%) than in highland (48%). The finding of the present study followed the similar pattern as 47.94% prevalence at Murree Wildlife Park that had higher elevation and 20.57% prevalence at Bahawalnagar Wildlife Park, which had lowest elevation levels among selected sites. Another study by Zamora-Vilchis *et al.* (2012) explored the relationship between the prevalence of endoparasitic species and the temperature, which indicated that a higher prevalence of parasites was found in areas with elevated temperatures compared to those with lower temperatures.

In the case of Bahawalnagar Wildlife Park, the prevalence of endoparasites was lower than that in Jallo wildlife park. This difference can be attributed to the favorable moist conditions that promote endoparasite outbreaks. Along with Murree Wildlife Park moist climatic conditions, the management conditions at Murree wildlife park were also suboptimal in comparison to the other two sites. Moreover, the captive birds at Murree Wildlife Park were older, and deworming was not done regularly, which could be a significant contributing factor to the higher infection rate observed at Murree Wildlife Park.

CONCLUSION

Coprological analysis from this study identified endoparasites from various phylogenetic classes in fecal sample (n=90) of *P. cristatus* from three geographically different sites of Punjab. High heterogeneity and prevalence of endoparasites were detected without apparent clinical symptoms of sickness indicative of subclinical sickness at all selected sites. Moreover, the endoparasitic occurrence and diversity varied significantly across the sites, with the highest rates of infestation observed at Murree wildlife park, followed by Jallo Wildlife Park and Bahawalnagar Wildlife Park. These variations were influenced by certain factors such as immunity of each captive bird, development of resistance to dewormers over time due to repeated use, managerial practices and climatic conditions of each site. However, the primary concern from this study was the intensity of coccidia (*Eimeria* sp.) prevalence at all selected sites.

DECLARATIONS

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IRB approval

The Committee on Animal Rights and Welfare, GC University Faisalabad, Pakistan approved this study (DZ/125/2019).

Ethical approval

The Committee on Animal Rights and Welfare, GC University Faisalabad, Pakistan approved this study (DZ/125/2019).

Statement of conflict of interest

The authors have declared no conflict of interest.

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