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Research Article

Effect of Endoparasites on Hematological Parameters of Captive Wood Green Pheasant (*Phasianus versicolor*) at Dhodial Pheasantry, Mansehra

Komal Hussain[1], Asma Kaleem[2], Muhammad Sohail[3]*, Muhammad Mujtaba[3], Muhammad Sajid[3], Muhammad Shoaib[3], Rameez Iqbal[3]

[1]Hazara University Mansehra, Mansehra District, Khyber Pakhtunkhwa, Pakistan; [2]Government Post Graduate College Mandian Abbottabad, Hazara University Mansehra, Mansehra District, Khyber Pakhtunkhwa, Pakistan; [3]Veterinary Research and Disease Investigation Center Abbottabad Khyber Pakhtunkhwa, Pakistan; [3]Regional Director, Veterinary Research and Disease Investigation Center Abbottabad Khyber Pakhtunkhwa, Pakistan.

Abstract | Pheasants are large bodied gallinaceous birds with an ornamental plumage having magnificent colors and shades that adds splendor and grandeur to its appearance. A brief study was conducted to find out the effect of Endo-parasites on hematological parameters of Wood Green Pheasant in captivity. In this study, total 200 samples were randomly collected from the cages of *Dhodial pheasantry*, Mansehra. Fecal samples of the Wood Green pheasant were analyzed to record the parasitic prevalence. Six parasitic species of the nematodes followed by two species of cestodes identified from faecal samples viz. *Ascaris* species (79%), *Strongyloides avium* (66%), *Capillaria* spp. (62%), *Heterakis* spp. (60%), *Syngamous trachea* (64%), *Tetrameres americana* (7%) and *Raillietina* spp.(15%), *Davianea proglottina* (5%) respectively. The overall prevalence of all these helminthic parasites was recorded as 90%. In the present research, there was no significant($P < 0.05$) association of Egg per gram (EPG) with sex of pheasents. Parasitic load has been strongly correlated to the hematological parameters i.e., Mean Corpuscular Haemoglobin concentration (MCHC), haemoglobiin (Hb), Mean Corpuscular Volume (MCV) and Total leukocyte count (TLC). A decrease in hematocrit, erythrocytes, and Hb values was observed ($P < 0.05$), while monocytes, eosinophils, heterophil/ lymphocyte increased ($P < 0.05$). The findings have educated about the impact of endo-parasites in clinically stable pheasant on selectedhematological parameters.

Keywords | Captivity, Endoparasites, Hematology, Pheasent, Egg per gram

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***Correspondence** | Muhammad Sohail, Senior Research officer: Veterinary Research and Disease Investigation Center Abbottabad Khyberpakhtunkhwa, Pakistan; **Email:** dr.msohail@ymail.com
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INTRODUCTION

Birds are one of the most fantastic creatures of the earth, and their diversity leads to a vibrancy of life (Sharma *et al.*, 2018). Pheasants are large bodied gallinaceous birds with an ornamental plumage having magnificent colors and shades that adds splendor and are considered as valuable biological environmental indicator (Shafiq and Saqib, 2011; Sailo, 2019). Taxonomically, they are representing the 'Phasianidae' family of order "Galliformes", which contains a large family of 52 species from 16 genera, with 51 surviving

species (Krupnick and Kress, 2003). Of these, 50 are native Asians, except the Congo peafowl, which is confined to Central Africaforests of the Democratic Republic of the Congo (Zaman, 2008).

The Himalayas are the highest and oldest mountain range in the world. About 80% of all Indian pheasant species are found in Himalaya (Selvan *et al*., 2013). The true Pheasants (*viz*., Monal, Kalij, Koklass, Cheer and the Western Horned Tragopan) of the Phasianinidae family from 5 separate genera are native to Himalaya, Hazara, Swat, Chitral, Murree Hills, Kashmir and some parts of the northern regions of Pakistan. They change their residential areas by the year as temperature increases and snowfall melts they move towards high altitudes and when the temperature decrease and snowfall increases, they shifted downwards (Shafiq and Saqib, 2011). According to the International Union for Conservation of Nature (IUCN, 1998) red list, over one third of the total pheasant species are legally reported at a risk of extinction from their naturalhabitat (Selvan *et al*., 2013). Out of 51 species documented, 3 are listed as Endangered (EN), 21 are classified as Vulnerable (VU), 26 are listed as Lower Risk (LR) and the remaining 1 specie referred as data deficient species (Fuller and Garson, 2000).

Dhodial Pheasantry, located on Karakoram Highway, founded in 1984 and is spread over an area of approximately five hectares at Dhodial in Mansehra District. The Pheasantry was developed for the general public and other aviculturists to gain experience in breeding Ring-necked Pheasants and other exotic species. The pleasantry has gained an international reputation where out of 52 pheasant species present in the world, the Dhodial Pheasantry has 32 species, making it the largest pheasantry in Asia. There are 400 cages currently to hold over 4000 birds. Prominent pheasant species in the pheasantry include species that are native to Pakistan (Koklass, Kalij, Monal, Cheer, and Tragopan) and exotic species like the Golden, Lady Amherst, and Reeves pheasant (Zaman, 2008).

Wood green pheasant is also known as the Japanese green pheasant. It is Japan's national bird (Bohl, 1964). Because of its magnificent and brightly colored males, wildlife enthusiasts are attracted to this group, and it is resilience to human invasion and its general accessibility of the unexperienced birder (Rahman *et al*., 2023).

Captive breeding plays a key role in revival of some species for which effective alternatives are not available in the short term. It is a framework by which efforts are made to breed threatened or endangered bird species in captivity so that they can be reintroduced into their natural habitats. Sometimes the population of a specie drops to such low numbers in the wild that it is almost certain to become extinct unless we intervene to save it (Ali *et al*., 2011).

In captive breeding these pheasents are more prone to be infested by endoparasites (nematodes, trematodes and cestodes) because they are enclosed in smaller area as compared to their natural habitats. These parasites are considered to be natural rivals who use the energy that their host creates and harm their actions and reproductive behavior, etc. Depending on age, immune system status and the burden of infection, parasites invade their host with some degree. Many endo-parasites have been found to feed into the host bowel or they may be connected to mucosal layer in the bowel where the blood or epithelial tissues of the host are drained by these parasites. Young parasites severely damage hosts, while well adapted and altered parasites do less damage to their hosts. These parasites not only alter the absorption of nutrients but also affect the hematological parameters. Blood serves as a pathological reflector of toxicants and other factors that areresponsible for disease in animals (Fudge, 2000). Therefore, understanding of blood components is a significant diagnostic aid for understanding of the status of pheasant. No data was available regarding occurrence of endoparasites in pheasents and their effect on blood components in hazara region.

Keeping in view the above facts, current study was designed to study the prevalence of endo-parasites, their effect on hematological parameters in captives Wood Green pheasant and to devise strategy for timely treatment and deworming schedule round the year.

MATERIALS AND METHODS

Study area

The study was carried out at Dhodial Pheasantry, Mansehra and samples were analysed at Veterinary Research and Disease Investigation Centre (VRDIC) Mandian, Abbottabad. The Mansehra District is situated in the north-eastern region of Khyber Pakhtunkhwa. The Mansehra region's flora and fauna is quite different and includes a great selection of interesting animals and plants (Shahi *et al*., 2015).

Sample collection

Fecal and Blood samples were collected from wood green pheasant reared at Dhodial Pheasantry, Mansehra and then brought to the Parasitology and Hematology laboratories respectively, of the VR and DIC Mandian, Abbottabad. Fresh fecal samples were on daily basis, stored in the plastic bags with 10% formalin as a preservative and placed in an ice bag (Zajac and Conboy, 2012). The blood samples of the Wood Green Pheasants were collected directly from

brachial vein with 3ml syringe and placed into EDTA containing vacutainers. The samples were placed inside ice box (Samour *et al.*, 2010) and then transported to Lab for further analysis.

Coprological examination

Quantitative method

Fecal samples of Wood green pheasant were sorted out in Parasitology lab by qualitative and quantitative method. Some common methods for separating eggs from fecal material includes direct smear, fecal flotation and sedimentation, others include McMaster respectively. The fecal examination makes it easy to know the diversity of parasites and their effects on the health of birds (Akinson *et al.*, 2009; Zajac and Conboy, 2012).

Qualitative method

This is the simplest form of microscopic fecal analysis for parasites, consisting of a small number of feces positioned directly on the slide of the microscope (Demalash *et al.*, 2016).

In this method, clean and dry glass slides were taken free of scratches. Small amount of feces was added to a drop of water and mixed gently with tooth pick. After covering it with cover slip the smear was examined under microscope for parasite eggs.

Quantitative method

McMaster egg counting method was used in order to obtain precise information with regard to severity of infection. The McMaster slide consists of two glass or plastic slides, which are connecting (by aquarium sement) two chambers of 0.15 volumes. Two (02g) of faces were weighed out and mixed with the 28 ml saturated sugar: salt solution, grinded, and sieved into dish. 12 ml solution was then added in the Centrifuge tube and centrifuged for 5 min. over 1500 rpm. Supernatant was taken with a pipette and transferred into chambers of the McMaster slide and examined at10X magnification. The EPG was calculated using following Formula (Zafar, 2008).

$$EPG = \frac{\text{Eggs counted (No's)}}{\text{Volume observed (ml)}} \times \frac{\text{Total volume (ml)}}{\text{weight of feces (gm)}}$$ (Zajac *et al.*, 2012)

Hematological examination

Hematology is also a beneficial diagnostic tool in veterinary medicine and can be used as physiological indicators. Hematological values are widely used as to the health of birds housed in cage systems to identify stress caused by different factors, such as environmental, nutritional and pathological aspects (Hrabčáková *et al.*, 2014). Complete Blood Count (CBC) was done by using Automated Hematological Analyzer (URIT-2900 Vet Plus).

Statistical analysis

Collected data was arranged in MS Excel and was analyzed through Chi square test for determination of significance values whereas, Box and Whisker plot was used for parasitic load (EPG). Hematological parameters were analyzed through General ANOVA and means were compared by Duncan's multiple range tests at a probabilitylevel ≤ 0.05. Statistical analytical software Statistics 8.1 was used for analysis.

RESULTS AND DISCUSSION

The present study was conducted on Wood Green pheasant at Dhodial Pheasantry, Mansehra. Total 200 (Male=60, Female=140) fecal samples were collected in clean polythene bags and examined at Veterinary Research and Disease Investigation Centre, Abbottabad.

Overall prevalence

Overall prevalence was calculated using simple percentage formula and was found that that 90% of wood green pheasant was found positive for worm infestation. The nematodes observed were*: Ascaris* spp. (79%), *Strongyloides avium* (66%), *capillaria* spp. (62%), *Heterakis* spp. (60%), *Syngamous trachea* (64%), followed by two species of cestodes viz., *Raillietina* spp. (15%), *Davianea proglottina* (5%) *Tetrameres americana* (7%). About 92% of male pheasants were affected while prevalence was lower in females (89%).

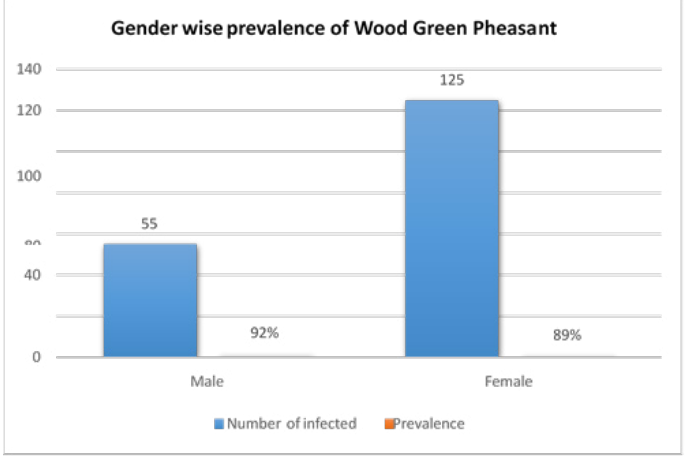

Figure 1: Gender wise prevalence of parasitic infection.

Gender wise prevalence

Prevalence of helminth parasites in male wood green pheasant

A total of 60 fecal samples of male wood green pheasants were examined, of which 55 (92%) were positive for one or more species of helminth parasites. In male pheasants 6 species of nematodes namely, *Ascaris* species, *Heterakis galliae*, *Capillaria* species, *Strongyloides avium, Syngamus trachea, Tetrameres americana* and two were cestodes namely, *Davainea proglottina*, *Raillietina* were seen. In

case of nematodes, highest prevalence rate was shown by *Ascaris* species (80%) and lowest was shown by *Tetrameres americana* (6%). While in case of cestodes the highest prevalence rate wasshown by *Raillietina* specie (13%) and lowest was shown by *Davainea proglottina* (1%).

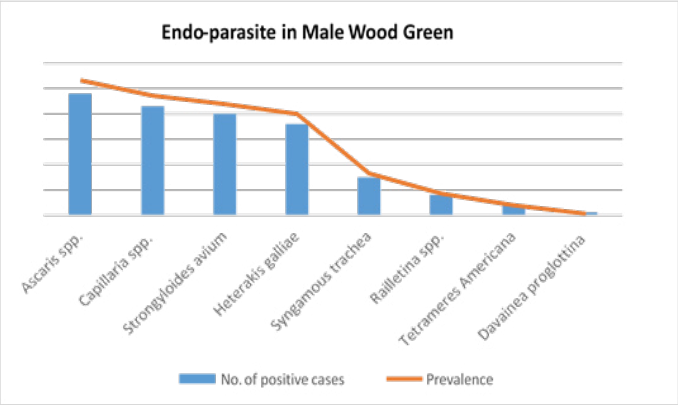

Figure 2: Prevalence in male wood green pheasant.

Prevalence of intestinal parasites in female wood green pheasant

A total of 50 fecal samples of male wood green pheasants were examined, of which 49 (98%) were found positive for one or more species of helminthe parasites. In female pheasants, 6 species of nematodes namely, *Ascaris*, *Heterakis galliae*, *Capillaria*, *Strongyloides avium*, *Syngamus trachea* and *Tetrameres Americana* while two were cestodes namely, *Davainea proglottina*, *Raillietina* species were seen. In case of nematodes, highest prevalence rate was shown by Ascaris spp (78%). and lowestwas shown by *Tetrameres Americana* (2%). While in case of cestodes the highest prevalence rate was shown by *Railletina* spp. (14%) and lowest was shown by *Davainea proglottina* (10%).

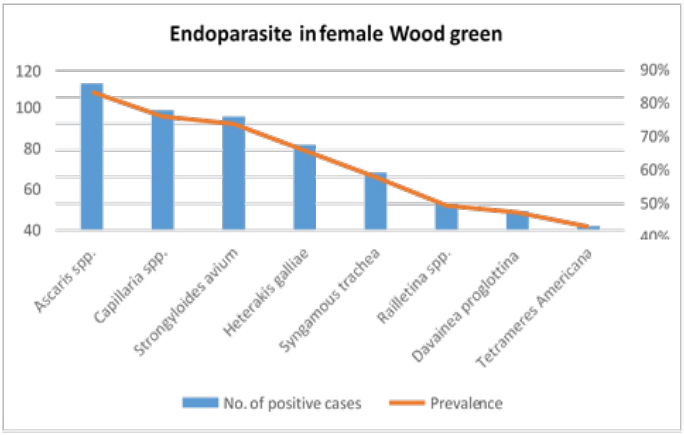

Figure 3: Prevalence in female wood green.

Effect of EPG on hematological parameters

Hematological parameters of Wood Green pheasant were studied in detail with respect to sex. Table shows that total Erythrocytes, Hemoglobin, Hematocrit, Mean corpuscular volume, Mean corpuscular Hemoglobin, Mean corpuscular Hemoglobin concentration, Granulocytes, Lymphocytes, Monocytes, Mean platelet volume, Platelet Distribution Width, Platelets, Red Blood Cell Distribution Width repeat precision, Red Blood Cell Distribution Width STDEV were not significantly ($p > 0.05$) affected by sex while White Blood cells were significant statistically ($p < 0.05$). In the current study, the effect of sex was non-significantly associated with hematological parameters.

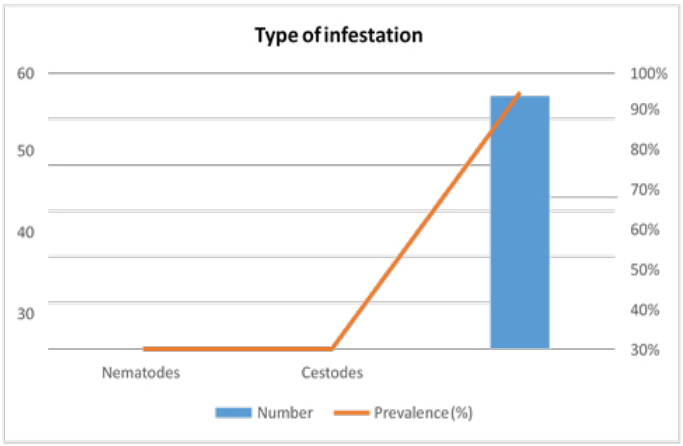

Figure 4: Type of infestation in female.

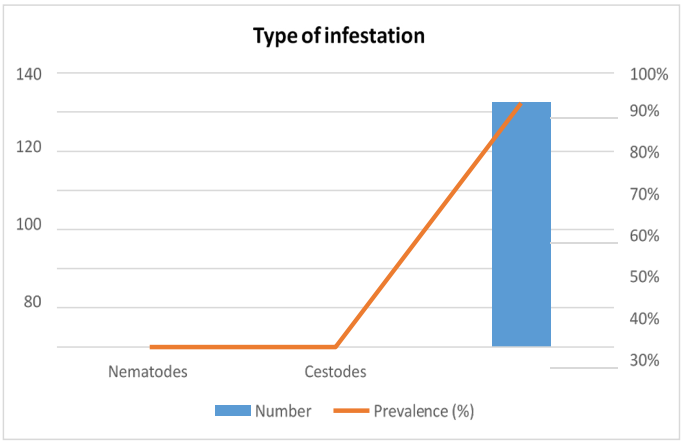

Figure 5: Type of infestation in male wood green pheasant.

Different superscript along the row indicate significance at p value ≤0.05The effect of Eggs per gram (EPG) were observed on the hematological profile of Wood Green Pheasant. The EPG was divided into three categories i.e., low EPG (≤ 500), Average EPG (≤ 1000). High EPG (> 1000).

The following parameters *viz.*, RBC, HB, HCT, MCV, MCH, MCHC, PDW, RDW- SD were significantly ($p < 0.05$) affected with increase in EPG while differences in parameters namely WBC, Gran, LYM, MID, MPV, PCT, PLT, RDW-CV were non-significant ($p > 0.05$). The erythrocytic indices i.e., MCV, MCH, and MCHC were significantly associated ($p < 0.05$) with gastrointestinal parasite.

Table 1: Hematology parameters for wood green pheasant with respect to sex.

Hematologyparameters	Normal ranges		Sex (Mean±SE)		P value
	Male	Female	Male	Female	
RBC (*10^6 /mm^3)	2.55±0.5	2.67±0.5	2.7±0.12[a]	2.8±0.2 [a]	0.6
Hb (g/dl)	14.5±0.5	13.7±0.5	21.9±0.89 [a]	20.3±1.2 [a]	0.3
HCT (%)	37-47	33-38	45.2±2.1 [a]	44.3±2.4 [a]	0.7
MCV (fl)	109-147	109-147	164.6±1.5 [a]	162.2±3 [a]	0.43
MCH (pg)	46.5±8.48	46.5±8.48	79.8±1.4 [a]	76.2±2.6 [a]	0.2
MCHC (g/dl)	28-36	28-36	48.6±0.6 [a]	46.5±1.5 [a]	0.16
WBC (10^9/mm^3)	28.8±8	19.9±4	20±1.3b	37.1±8.5a	0.00
Gran (G/L)	2.42-6.38	3.06-6.38	4.2±0.3 [a]	3.9±0.37 [a]	0.5
Lym(10^9/ L)	8.7-18.12	8.7-21	9.2±0.5 [a]	9.8±0.6 [a]	0.4
MID (%)	1.1-3.0	1.7-3.5	5.4±0.22 [a]	5.2±0.25 [a]	0.6
MPV (fl)	13.5-15.9	13.5-15.9	16.4±0.3 [a]	16.5±0.32 [a]	0.8
PCT	0.1-0.5	0.1-0.6	0.44±0.01 [a]	0.44±0.03 [a]	0.9
PDW (%)	9.0-17.0	9.0-17.0	10.8±0.28 [a]	11.1±0.76 [a]	0.5
PLT(10^3/mm^3)	24.8-34.1±12	17.0-27.1±6	27.0±14.1 [a]	27.3.4±27.3 [a]	0.9
RDW-CV (%)	15-16	15-16	13.5±0.3 [a]	14.8±0.6 [a]	0.06
RDW-SD (fl)	39-56	39-56	73.1±1.6 [a]	76.4±2.4 [a]	0.27

Table 2: Hematology parameters for wood green pheasant with respect toEPG.

Hematology parameters	EPG (Mean±SE)			P value
	Low EPG	Average EPG	High EPG	
RBC	3.1±0.14[a]	2.8±0.2 [b]	2.1±0.2 [c]	0.03
Hb	23.1±1.1 [a]	19.3±1.2[b]	14.2±1.9[c]	0.01
Hct	48.1±2.2 [a]	40.4±0.5[b]	39±4[b]	0.04
MCV	167.5±1.2 [a]	161.7±2.9 [a]	161.2±2.9 [a]	0.12
MCH	74.5±1.1 [a]	68.9±3 [b]	67.6±1.1 [b]	0.03
MCHC	48±0.6 [a]	47.7±1.5[a]	36.4±0.7[b]	0.04
WBC	23.1±2.37 [a]	26.1±5.5 [a]	28.4±8.3 [a]	0.78
Gran	24.1±0.38 [a]	22.3±0.4[a]	20.8±0.5[a]	0.06
Lym	70.5±0.5 [a]	71.9±0.6 [a]	74.2±1.1 [a]	0.46
MID	5.3±0.2 [a]	5.5±0.2 [a]	4.9±0.47 [a]	0.4
MPV	16.2±0.2 [a]	16.6±0.4 [a]	16.5±0.6 [a]	0.7
PCT	0.4±0.01 [a]	0.4±0.03 [a]	0.4±0.03 [a]	0.9
PDW	10.4±0.2[b]	11.9±0.6[a]	9.9±0.5[b]	0.02
PLT	269.7±17.3[a]	284.9±23.2[a]	249.8±32.3[a]	0.6
RDW-CV	14.4±0.3 [a]	13.6±0.5 [a]	13.6±0.6 [a]	0.4
RDW-SD	78±1.7[a]	71±2.4[b]	72.8±0.3[b]	0.07

Different superscript along the row indicate significance at p value ≤0.05

Risk factors for helminthiasis

The qualitative analysis of fecal samples in Wood Green pheasant were studied with respect to gender (table). Gender wise EPG was recorded higher for male (3750) as compared to female (1750), while IQR was higher for female (637.5) followed by male (425) but the difference was non-significant (p>0.05).

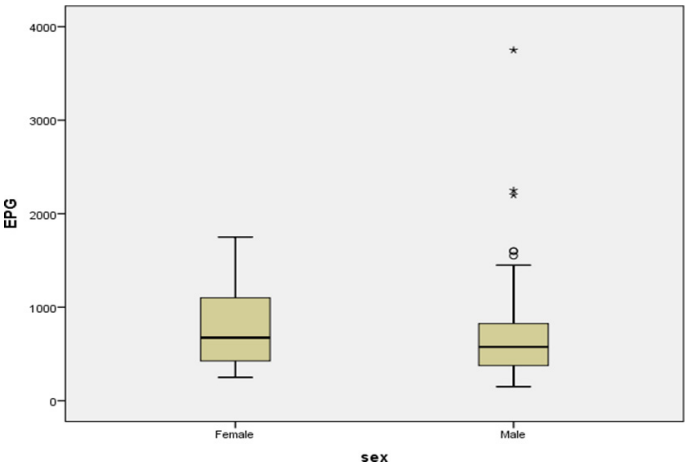

Figure 6: Box and whisker plot showing effect of sex on EPG of wood green pheasant.

DISCUSSION

Parasitic infections caused by helminths represent a major health concern in both free-living and farm-reared pheasants, leading to reduced productivity, compromised immunity, and increased susceptibility to secondary infections. Numerous studies worldwide have documented a wide diversity of gastrointestinal parasites affecting captive and semi-captive avian species (Edosomwan and Igetei, 2018; Akram *et al.*, 2019; Permin and Hansen, 2020).

In the present study, the overall prevalence of gastrointestinal

parasites in Wood Green Pheasants was recorded as 90%, which is considerably higher than the prevalence of 35.5% reported by Nnadi and George (2010). This elevated prevalence may be attributed to management practices, environmental contamination, continuous exposure to infective stages, and the absence of regular deworming programs in captive pheasant populations (Soulsby, 2012; Permin and Hansen, 2020). Qualitative examination revealed the presence of multiple gastrointestinal helminths, with nematodes being the most prevalent group, followed by cestodes. This finding contrasts with the observations of Puttalakshmamma *et al.* (2008), who reported cestodes as the dominant helminths in avian species. Such variations may arise due to differences in geographical location, host species, feeding habits, climatic conditions, and intermediate host availability (Ashraf *et al.*, 2021).

Among nematodes, Ascaridia spp. showed the highest prevalence (79%), indicating its dominance in the study population. This observation aligns with earlier reports by Matur *et al.* (2010), Ngongeh *et al.* (2014), and Ashraf *et al.* (2021), who identified *Ascaridia* as the most frequently encountered nematode in galliform birds. The high prevalence of *Ascaridia* spp. may be linked to their direct life cycle and the prolonged survival of eggs in the environment, facilitating rapid transmission in confined rearing systems (Permin and Hansen, 2020).

Eggs of *Capillaria* spp. were also frequently detected, corroborating previous findings by Pradeep *et al.* (2017) and Khan *et al.* (2022), who emphasized the pathogenic potential of capillariasis in captive birds. Additionally, the significant prevalence of *Heterakis* spp. observed in this study supports earlier findings by Parsani *et al.* (2012) and Ashraf *et al.* (2021), highlighting its widespread distribution in pheasants and other galliform birds.

Sex-wise analysis revealed a higher parasitic burden, egg per gram (EPG), and interquartile range (IQR) in females compared to males. These findings are consistent with Fadunsin *et al.* (2019) and Khan *et al.* (2022), who suggested that hormonal influences, reproductive stress, and higher nutritional demands may predispose female birds to increased parasitic infections.

Hematological parameters and plasma metabolite levels are valuable indicators of physiological and health status and form an essential component of disease diagnosis in avian species (Campbell and Ellis, 2007; Thrall *et al.*, 2019). In the present study, most hematological parameters were not significantly affected by sex, except for parasitic load (EPG). White blood cells (WBCs) play a critical role in immune defense, while carotenoids contribute to immune modulation (Osman *et al.*, 2004; Sladin, 2003). The significantly higher WBC count observed in females (37.1 ± 8.5) compared to males (20 ± 1.3) is consistent with the findings of Kececi *et al.* (2011) and may reflect heightened immune responses due to increased parasitic exposure.

Although reference intervals for hematological parameters have been established for many domestic avian species, limited information is available for pheasants (Campbell and Ellis, 2007). In this study, sex had a non-significant effect on most hematological indices, including total erythrocyte count, hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), plateletcrit (PCT), and platelet count (PLT). However, total erythrocyte count was slightly but significantly higher in females (2.8 ± 0.2) than males (2.7 ± 0.12). Hemoglobin and packed cell volume (PCV) values were marginally higher in males, though the differences were statistically non-significant. Similar trends have been reported by Ravindra *et al.* (2003) and Kececi *et al.* (2011).

Furthermore, the effect of parasitic burden (EPG) on hematological parameters was evident in the present study. Birds were categorized into low (≤ 500), moderate (≤ 1000), and high (> 1000) EPG groups. Increasing EPG levels significantly affected erythrocytic parameters including RBC count, Hb, hematocrit (HCT), mean corpuscular volume (MCV), MCH, MCHC, platelet distribution width (PDW), and red cell distribution width-standard deviation (RDW-SD) ($p < 0.05$). In contrast, leukocytic parameters such as WBC, lymphocytes, granulocytes, MID cells, mean platelet volume (MPV), PCT, PLT, and RDW-CV showed no significant variation ($p > 0.05$). These findings indicate that gastrointestinal helminth infections primarily impair erythropoiesis and red cell indices, leading to anemia and reduced oxygen-carrying capacity, as previously reported by Soulsby (2012), Thrall *et al.* (2019), and Khan *et al.* (2022).

CONCLUSION

It is concluded from the coprological study of investigation that the *Ascaris* species (75%) have highest rate of prevalence while *Tetrameres americana* (7%) have the lowest rate of prevalence.

The results revealed that other haematological parameters were not significantly ($p>0.05$) affected by sex except White Blood cells which were significant statistically ($p< 0.05$).

The helminth parasites have a significant effect on their blood parameters followed by their health, which further decreases their development, so that accurate diagnosis,

treatment and prevention methods are required in order to avoid their effects on pheasant health.

RECOMMENDATIONS

It is recommended that basic hygienic measures should be adopted in feeding and watering. Proper biosecurity should be adopted. Balanced nutritive food should be provided. Regular routine deworming and vaccination should be performed.

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AUTHOR'S CONTRIBUTION

M. Sohail, M. Sajid and M. Mujtaba conceived the idea of study. K. Hussain and A. Kalim collected samples and analysed. M. Sohail supervised the sample analysis in laboratory. M. Sajid helped in sample collection. R. Iqbal, A. Kalim, M. Sohail and K. Hussain did the write-up and data analysis.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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