











## PREVALENCE OF HAEMOPARASITES AND HEMATOLOGICAL PARAMETERS IN PIGEONS OF DISTRICT FAISALABAD, PAKISTAN

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### ABSTRACT

The commonly occurring haemoparasites in pigeons include *Plasmodium*, *Leucocytozoon* and *Haemoproteus* which cause decrease in production and performance of the birds. Due to the dearth of knowledge on prevalence of these parasites in pigeons in Pakistan, current survey-based study was planned to assess the prevalence of above-mentioned parasites in pigeons in selected rural areas of District Faisalabad, Punjab, Pakistan. The morphological identification of parasites was done under microscope by comparing it with standard keys. Out of 384 samples, 56.51% were found positive for haemoparasites. According to town-wise distribution, Jinnah town, Iqbal town, Madina town and Lyallpur town prevalence was 61.24, 48.86, 55.56, and 59.26%, respectively. According to genus-wise distribution, 48.14, 48.21, and 64.92% prevalence of mixed infection, genera *Plasmodium*/*Haemoproteus* and *Leucocytozoon* were observed, respectively. The prevalence was found high in female birds (65.9%) as compared to male birds (51.18%). The prevalence was higher in Cheena (69.02%) and Golden breeds (76.57%) as compared to Sialkoti (32.89%) and Dub wali (37.17%). The prevalence was found higher in squabs (74.15%) as compared to adults (49.6%) and growers (52.56%), in scavengers feeding pattern (77.31%) as compared to feeders (35.78%), and in open coop type (76.68%) as compared to closed coop type (36.12%). The values of Hb, PCV, and RBC's count were lower, while WBC's counts were higher in infected pigeons. The values of uric acid, creatinine, albumin and bilirubin were lower while gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST) and glucose were higher in infested birds as compared to non-infested birds. The study indicated the impact of haemoparasites on the hematological parameters of the birds and the data generated could help in formulating effective control strategies against haemoparasites particularly *Plasmodium*, *Leucocytozoon* and *Haemoproteus*.

**Keywords:** Hematology, Haemoproteus, Leucocytozoon, Plasmodium

Article History (2023-0133) || Received: 13 May 2023 || Revised: 18 Jun 2023 || Accepted: 21 Jun 2023 || Published Online: 26 Jun 2023

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### 1. INTRODUCTION

Pigeon rearing has grown as good industry and has created jobs for many people in Pakistan. These precious birds are reared for various purposes like gaming, gambling, fancy exhibition at national shows and competitions. Due to its distinct flight, it has been sold in high prices. Its relationship with man is very old and has been domesticated by for hundreds of years. Pigeons also played a significant role in World War I and II. They have also been used as lab animals for the detection of fowl cholera. Pigeon feed on a wide range of food items, including grains, slugs, earthworm, and insects, which in many instances may carry infective stage of haemoparasites (Capasso et al. 2023).

Pigeons are present across the globe, but apart from the Polar Regions, species of wild pigeons i.e., *Columba oenas*, *C. livia* and *C. palumbus* are inhabitant of various demographic areas (Poulsen et al. 2000). The most common pigeon's breeds in Pakistan are Teddy, Chapry, Dub wali, Golden, Banka, Sialkoti and Cheena, which are reared for several purposes (Villalva-Pasillas et al. 2020; Sadeef et al. 2015).

Pigeons have long been subjected to intense ornithological and parasitological study. The impact of blood parasites on avian host fitness has been the focus of numerous studies. The annual variability of these parasites and their impact on avian host fitness are still very much unknown (Adeyemo et al. 2013).

**Citation:** Nadeem M, Khan MK, Mughal MAS, Abbas RZ, Imran M, Sindhu ZD, Zafar A, Abbas Z, Jamil T and Latif M, 2023. Prevalence of haemoparasites and hematological parameters in pigeons of district Faisalabad, Pakistan. *Agrobiological Records* 12: 76-82. <https://doi.org/10.47278/journal.abr/2023.017>

Pigeons have a very close relationship with domesticated chickens. It is well documented that haemoparasites of chicken and pigeons are similar. So, pigeons can be the carrier of many parasitic infections and may transmit haemoparasites to domestic chicken (Włodarczyk et al. 2022).

Poultry industry has come across many health problems like viral, bacterial and parasitic infections along with manage mental and malnutrition problems (Bachaya et al. 2015). In management systems, where chickens are not confined but also have open access to outdoor areas; that's why there are great chances of getting parasitic infection, especially with blood parasites. The occurrence of blood parasites is more in hot areas birds (De Aguiar et al. 2021).

The most common haemoparasites of pigeons are *Haemoproteus*, *Plasmodium* and *Leucocytozoon*. Studies have shown high prevalence of haemoparasites in wild and domestic pigeons. These haemoparasites can cause significantly high mortality in pigeon species (Elahi et al. 2014; Cepeda et al. 2019). Mostly vectors i.e., black flies, mosquitoes, louse and biting midges play an important role in transmission of these parasites. Some of these may cause anemia and lead to subclinical infection (Habibu et al. 2022).

These haemoparasites have been reported in pigeons globally with a relatively high prevalence and have also been reported in chicken from district Layyah, Pakistan (Naqvi et al. 2017). The presence of very limited published data regarding occurrence of these parasites in pigeons indicated a dire need for conducting this study to estimate the prevalence of *Haemoproteus*, *Leucocytozoon* and *Plasmodium* from District Faisalabad, Punjab, Pakistan. Risk factors associated with the prevalence of haemoparasites and the effects on host serum profile were also determined.

## 2. MATERIALS AND METHODS

### 2.1. Ethical Statement

The ethical approval to conduct this research was taken from Directorate of Graduate Studies, University of Agriculture, Faisalabad. The approval letter No. DGS/7034-37 was signed by Director Graduate Studies.

### 2.2. Study Area

The blood samples were collected from selected regions of Faisalabad i.e., Jinnah town, Madina town, Iqbal town and Lyallpur town. The further processing of samples was done at Epidemiology Laboratory, Department of Parasitology, University of Agriculture, Faisalabad, Pakistan. Faisalabad is situated 186m above the sea level at coordinates of 31°15'0"N 73°03'0"E and belongs to semi-arid climate having very hot and humid summers and dry cool winters. The average temperature during summer is 26.9-40.5°C.

### 2.3. Sample Size Estimation

Sample size was estimated by Thrusfield (2018) formula by taking 50% expected prevalence, 5% desired precision and 95% confidence interval. A total of 384 blood samples were screened for haemoparasites presence in pigeons. The calculated samples were taken randomly with different sex and age groups. Ages of pigeons were assessed by xiphoid cartilage flexibility and crown size and correlate it with the information from the owner. Depending on age, pigeons were divided into squabs, growers and adults.

### 2.4. Collection of Blood Samples

384 blood samples were taken from wing veins of pigeon using sterile needles (22-27gauge) from selected towns of Faisalabad to examine for the presence of avian blood parasites. Preservation of blood samples were done in 3ml EDTA coated vacutainer (ATLAS-LABOVAC) with slide preparation on the spot. The samples were stored at 4°C in an ice box and taken to Epidemiology Laboratory, Department of Parasitology, University of Agriculture, Faisalabad, for further processing.

### 2.5. Parasitological Examination

Haemoparasites in collected blood samples were identified by thin blood smear. Briefly, after air drying the smear absolute methanol was used to fix the smear and stained by Giemsa staining method. Extra stain was washed by rinsing with distill water followed by air drying. Based on morphology, the parasites were examined under microscope at high magnification (40x and 100x).

### 2.6. Hematological Parameters

Hematological parameters were determined by using appropriate methods like, microhematocrit method for packed cell volume (PCV) and Sahli's apparatus for hemoglobin (Hb) level. Erythrocytes and leukocytes were counted by using hemocytometer chamber in a 1:200 dilution of blood in Natt and Herrick's solution (Natt and Herrick 1952).

## 2.7. Statistical Analysis

The variables among different categories of determinants were compared using odd's ratio and multiple logistic regression analysis. The hematological differences between infected and non-infected pigeons were evaluated using Tukey's test. All these analyses were performed in SAS software by keeping 5% absolute precision (Naqvi et al. 2017).

## 3. RESULTS

### 3.1. Prevalence of Haemoparasites

Briefly 384 blood samples were collected from pigeons of district Faisalabad in order to detect the prevalence of haemoparasites. 217 samples (56.51%) were found positive for haemoparasites i.e., *Plasmodium*, *Hemoproteous* and *Leucocytozoon*. The overall prevalence of parasites for Jinnah town, Iqbal town, Madina town, Lyallpur town, was 61.24, 48.86, 55.56 and 59.26% respectively, showing comparatively high prevalence in Jinnah and Lyallpur as given in Table 1.

Regarding sex-wise prevalence, 65.9% females showed hemoparasitic infestation (163/247) as compared to 51.18% males (70/137). In age related groups, 49.6% adult birds (69/139), 52.56% grower birds (82/156) and 74.15% squabs (66/89) were found infected with haemoparasites. Leucocytozoon prevalence was found higher (64.92%) than Haemoproteus/Plasmodium (48.21%) and mixed infection (48.14%) in all towns. Breed wise prevalence of haemoparasites was considerably high in Golden (76.57%) and Chenna (69.02%) than Dub Wali (32.89%) and Sialkoti (32.89%) ( $P < 0.05$ ), respectively. The prevalence of blood parasites was found considerably higher in pigeons kept in open coops (76.68%) than those of closed (36.12%). Scavenger pigeons have significantly more prevalence (75%) than that of close type feeding pattern (37.2%).

### 3.2. Relative Hematological Parameters

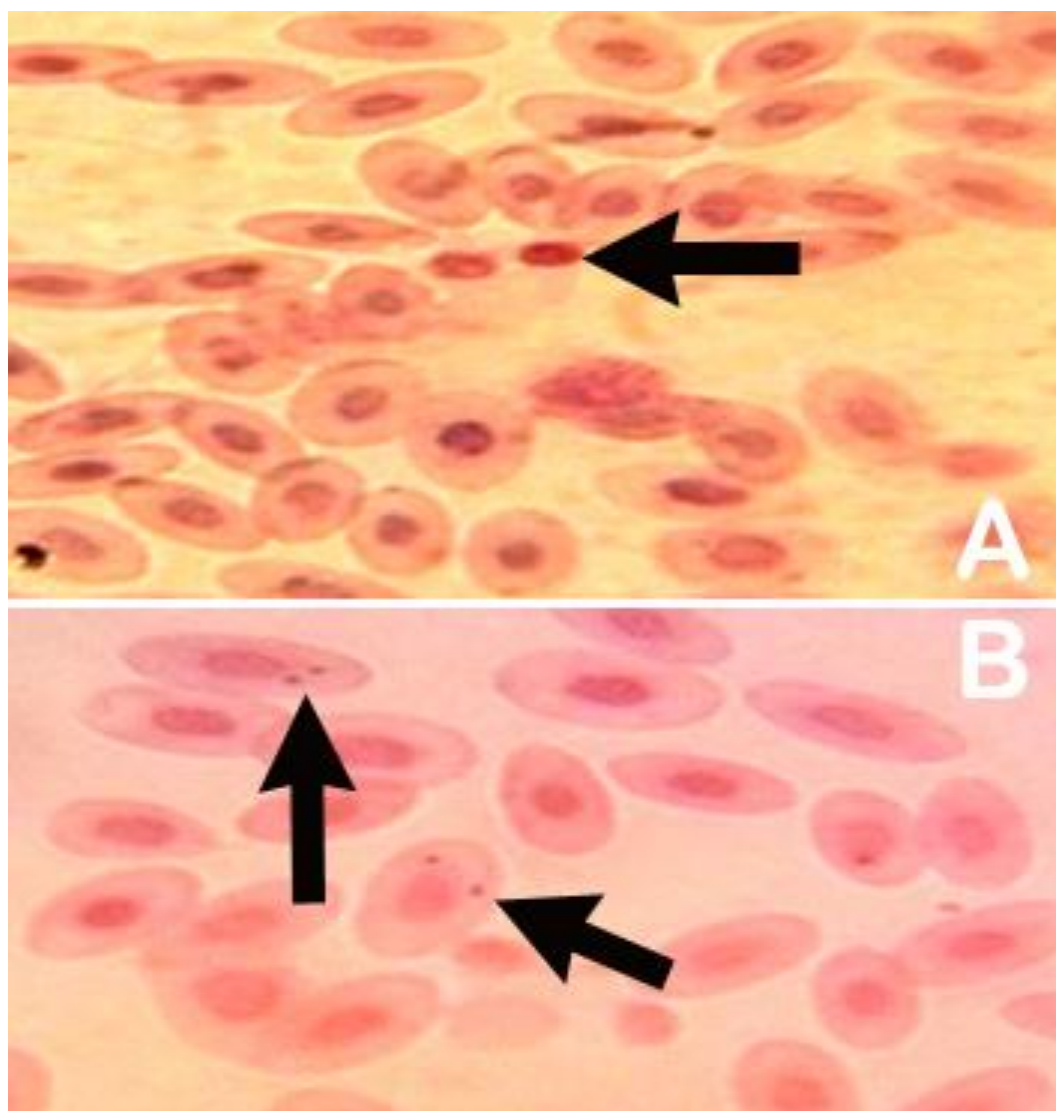
Hematological profile including WBC's and RBC's count, PCV and Hb level were observed in total 40 infected versus non-infected pigeons. The RBC's count ( $2.99 \times 10^6/\text{mL}$ ;  $P = 0.017$ ), Hb level (9.77g/dL;  $P = 0.011$ ) and mean PCV value (34.01%;  $P = 0.001$ ) were found significantly lower in infected birds as compared to non-infected birds. Alternatively, WBC's counts were higher ( $P = 0.008$ ) in infected birds (25.01/mL) than those of non-infected birds (21.05/mL) as mentioned in Table 2.

### 3.3. Comparative Serum Profile in Infected and Non-Infected Pigeons

In infected birds, mean GGT value (4.640IU/L), ALT (36.47IU/L), AST (148.35IU/L), Albumin (0.717mg/dL) and Creatinine (0.33mg/dL) were found higher than non-infected birds. Alternatively, in infected birds Bilirubin (0.087mg/dL) and Uric acid (3.35mg/dL), were found lower than non-infected birds as mentioned in Table 3.

**Table 1:** Prevalence of haemoparasites in pigeons from selected regions of District Faisalabad

Variables	Categories	Samples	Positive	Prevalence	Confidence Interval	Odds Ratio (OR)	P-value
Towns	Jinnah	98	61	61.24	1.0063-3.23	1.8057	0.0476
	Iqbal	88	42	48.86	-	-	-
	Madina	90	50	55.56	0.76-2.46	1.369	0.2965
	Lyallpur	108	64	59.26	0.90-2.81	1.5931	0.1079
Age	Adult	139	69	49.6	-	-	-
	Grower	156	82	52.56	0.71-1.77	1.1242	0.6161
	Squab	89	66	74.15	1.63-5.19	2.9112	0.0003
Gender	Male	137	71	51.18	-	-	-
	Female	247	163	65.9	1.17-2.76	1.8038	0.0067
Parasite Genera	Leucocytozoon	191	124	64.92	1.17-3.37	1.9931	0.0104
	Plasmodium/Haemoparasite	112	54	48.21	0.56-1.77	1.0027	0.9928
	Mixed	81	39	48.14	-	-	-
Pigeon Breeds	Cheena	110	74	69.02	1.87-6.62	3.4259	0.0001
	Dub Wali	80	30	37.5	-	-	-
	Sialkoti	77	35	45.45	0.73-2.62	1.3889	0.3123
	Golden	117	86	76.57	2.50-8.51	4.6237	0.0001
Coop Type	Open	193	148	76.68	3.72-9.07	5.8151	0.0001
	Close	191	69	36.12	-	-	-
Feeding Type	Feeders	190	68	35.78	-	-	-
	Scavengers	194	150	77.31	3.90-9.57	6.1163	0.0001



**Fig. 1:** Giemsa-stained blood smears of pigeons showing blood parasites including intracellular Leucocytozoon (A), and Haemoproteus/Plasmodium (B) as indicated by arrows. 1000X.

**Table 2:** Relative hematological parameters of pigeons from selected regions of District Faisalabad

Groups	PCV	Hemoglobin	RBC's	WBC's
Infected	34.01±4.01 <sup>a</sup>	9.77±0.97 <sup>a</sup>	2.99±0.22 <sup>a</sup>	25.01±2.05 <sup>a</sup>
Non infected	42.22±2.22 <sup>b</sup>	14.06±1.59 <sup>b</sup>	3.99±0.42 <sup>b</sup>	21.05±1.55 <sup>b</sup>

Values (Mean±SE) sharing different letters in each column are statistically significant ( $P<0.05$ ).

**Table 3:** Comparative serum profile of infected and non-infected Pigeons from selected regions of District Faisalabad

Parameters	Units	Groups	
		Non-Infected	Infected
Gamma-glutamyl transferase	IU/L	1.635±0.35 <sup>a</sup>	4.640±0.67 <sup>b</sup>
Alanine transaminase	IU/L	21.98±3.5 <sup>a</sup>	36.47±4.5 <sup>b</sup>
Aspartate transaminase	IU/L	80.57±5 <sup>a</sup>	148.35±9 <sup>b</sup>
Uric acid	mg/dL	5.63±0.67 <sup>a</sup>	3.35±0.5 <sup>b</sup>
Creatinine	mg/dL	0.357±0.02 <sup>a</sup>	0.338±0.001 <sup>a</sup>
Glucose	mg/dL	346±15 <sup>a</sup>	357±17 <sup>a</sup>
Albumin	mg/dL	0.467±0.001 <sup>a</sup>	0.717±0.002 <sup>b</sup>
Bilirubin	mg/dL	0.2486±0.002 <sup>a</sup>	0.087±0.001 <sup>b</sup>

Values (Mean±SE) sharing different letters in a row differ significantly ( $P<0.05$ ).

**Citation:** Nadeem M, Khan MK, Mughal MAS, Abbas RZ, Imran M, Sindhu ZD, Zafar A, Abbas Z, Jamil T and Latif M, 2023. Prevalence of haemoparasites and hematological parameters in pigeons of district Faisalabad, Pakistan. *Agrobiological Records* 12: 76-82. <https://doi.org/10.47278/journal.abr/2023.017>



#### 4. DISCUSSION

The prevalence of haemoparasites in pigeons was reported as 57% in the present study. Similarly, high prevalence of 79.1% was documented in Africa (Lutz et al. 2015) and 70 % in Iraq (Hasson 2015). In various other studies, low prevalence was recorded like 54.67% in Bangladesh (Dey et al. 2008), 42% in Philippine (Silva-Iturriza et al. 2012), 34% in South Korea (Ishtiaq et al. 2007) and 29.5% in Bangladesh (Elahi et al. 2014). The variations in prevalence of haemoparasites between previous and current studies may be due to geographical distributions, weather differences, and other determinants.

The prevalence of *Lecocytozoon* spp. was reported as 64.15% in pigeon in current study. The prevalence of haemoparasites was reported as 48% in pigeon from Iran (Samani et al. 2013) and 23.3% in Bangladesh (Islam et al. 2013). In contrast, lower prevalence of blood parasites was reported in various areas like 20% in Mymensingh district of Bangladesh (Dey et al. 2008), 21% in Bursa Region and 19.9% in Madagascar region (Raharimanga et al. 2002), 14% in central Philippine islands (Silva-Iturriza et al. 2012), 5% in Iran (Radfar et al. 2012) and 5% in Equatorial Guinea (Sehgal and Valkiunas 2006). In another study, higher prevalence (50.9%) of haemoparasites was documented in pigeons from different areas of Mymensingh and Netrokona districts of Bangladesh (Islam et al. 2013).

In the present study, the occurrence of *Hemoproteous/Plasmodium* spp. was reported as 48.12% in domestic pigeons. Similar consequences were documented for haemoparasites occurrence in Mauritius i.e., 30% in pink pigeons and 24% in pigeons of different areas of Mymensingh district of Bangladesh (Swinerton et al. 2005), 24.4% in mallard ducks in Washington (Dey et al. 2010) and 20% in wood ducks in Atlantic Flyway (Senlik et al. 2005). High prevalence of haemoparasites i.e., 58.25 and 43.63% in pigeon from Khulna and Chittagong (Islam et al. 2014), 60% in mallard ducks from Mymensingh region of Bangladesh (Dey et al. 2008), 91.2% in pintail ducks from Canada (Bennett 1987) and 86% in ducks in Mackenzie Delta and Alberta (Synek et al. 2016) have been reported. Alternatively, lower prevalence of haemoparasites i.e., 2% in pigeons from Khorasan province of Eastern Iran (Elahi et al. 2014), 3% in pigeons from Zimbabwe (Borji et al. 2011), 0.6% in ring-necked duck from wetlands of Bangladesh (Permin 1998) and 9.2% in birds from Florida (Scaglione et al. 2015) have also been reported.

In the present study, the prevalence of extra and intracellular spp. of blood parasites was reported as 48.12% in domesticated pigeon. Similarly, high prevalence of 58.25% have been documented in Khulna and Chittagong region of Bangladesh (Islam et al. 2014), 50% in eastern Iran (Borji et al. 2011) and 57% in Ankara District, Turkey (Gicik and Arslan 2001). Alternatively, low prevalence of 20% (Dey et al. 2010), 19.9% (Rukambile et al. 2020) and 24% (Samani et al. 2013) have also been documented.

In present study, the prevalence of blood parasites was found considerably ( $P < 0.05$ ) high in open rearing pigeons (76.68%) as compared to close rearing pigeons (36.12%). Similarly, the infection rate of 62.79 and 57.65% have been reported in open and close rearing system from India (Gupta et al. 2011) and 62.5 and 52.6% from Turkey (Gicik and Arslan 2001), respectively. Alternatively, low prevalence was reported in chicken and pigeons from different regions of Bangladesh (Dey et al. 2008). However, no significant difference was observed among open and close housed ducks in Missouri (USA) (O'Dell and Robbins 1994) and in Atlantic Flyway (Thul et al. 1980). The exact cause of high prevalence of haemoparasites infection in the open housed pigeons cannot be elucidated. It may be hypothesized that open reared pigeons are more prone to insect vectors and the bird's immune system is being suppressed by higher level of prolactin and progesterone which makes the bird more prone to any infection (Galosi et al. 2022).

In present study, the prevalence of haemoparasites was found significantly ( $P < 0.05$ ) high in squab (74.15%) than younger (52.56%) and adults (49.6%). Similarly, high prevalence of 69% and 31% from Bursa region of USA (Senlik et al. 2005) and 63.5% and 47.5% from Turkey have been reported in squabs and younger birds, respectively. Alternatively, low prevalence of 33.33 and 3.51% from Mymensingh and Netrokona districts of Bangladesh (Islam et al. 2013) and 18 and 2% from Missouri region of USA (O'Dell and Robbins 1994) have been reported in adult and young birds of pigeon and wood ducks, respectively. The high prevalence in squabs may be due to following reasons: (1) developing immune system, (2) less feathers on the body increasing the biting area for vectors, and (3) thin skin facilitating the mouth parts penetration.

#### 5. Conclusion

In this study the prevalence of haemoparasites i.e., *Leucocytozoon*, *Plasmodium* and *Haemoproteus* in pigeon were evaluated along with their impact on hematological parameters. The overall prevalence of parasites was found high in study area. Cheena and Golden breeds were found to have more haemoparasites prevalence. Regarding

feeding type, open coop type and scavengers got more infection. Infected pigeons were found to have low levels of hemoglobin, packed cell volume and red blood cells and high level of white blood cells.

## Authors Contribution

Muhammad Nadeem conducted the research under supervision of Muhammad Kasib Khan, Rao Zahid Abbas and Zia ud Din Sindhu. Muhammad Adnan Sabir Mughal completed the write up process in collaboration with Arsalan Zafar and Mehroz Latif. Muhammad Imran and Tariq Jamil helped to analyze the data by using various statistical designs. Zaheer Abbas proofread the manuscript which is finally approved by Muhammad Kasib Khan.

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**Citation:** Nadeem M, Khan MK, Mughal MAS, Abbas RZ, Imran M, Sindhu ZD, Zafar A, Abbas Z, Jamil T and Latif M, 2023. Prevalence of haemoparasites and hematological parameters in pigeons of district Faisalabad, Pakistan. *Agrobiological Records* 12: 76–82. <https://doi.org/10.47278/journal.abr/2023.017>

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