

Study Of Blood Parasites In Gallus Gallus Domesticus (Linnaeus, 1758) In Gulberg Town, Karachi.

Shazia Nisar¹, Rakhshinda Khurram^{2*}, Umm E Kulsoom³, Samina Arif⁴, Saima Siddiqui⁵

¹Federal Urdu University Arts Science and Technology, Karachi, Pakistan, HANDS-Institute of Development Studies, Karachi, Pakistan, shazia.nisar@fuuast.edu.pk

²*Federal Urdu University Arts Science and Technology, Karachi, Pakista, HANDS-Institute of Development Studies, Karachi, Pakistan , Khurramrakhshinda.khurram@gmail.com

³Federal Urdu University Arts Science and Technology, Karachi, Pakistan, Kulsoomikram26gmail.com

⁴ Federal Urdu University Arts Science and Technology, Karachi, Pakistan, Samina_arif18@hotmail.com

⁵Federal Urdu University Arts Science and Technology, Karachi, Pakistan, Ssaimasiddiqui7@gmail.com

*Corresponding Author: Rakhshinda Khurram

*Federal Urdu University Arts Science and Technology, Karachi, Pakista, HANDS-Institute of Development Studies, Karachi, Pakistan , Khurramrakhshinda.khurram@gmail.com

Abstract:

There were different blood parasites in avian blood. Different blood parasites were observed in Gallus *gallus domesticus*. We observed 50 blood samples of *Gallus gallus domesticus* in Gulbergtown. 35 samples were infected with different blood parasites and 15 samples were uninfected in which 5 samples of female and 10 samples of males. In 24 samples of female chickens (70%) infections were noticed and in males 11 samples (30%) infection of haemoparasites were noticed. In infected samples of female chicken, we observed 3 different haemoparasites; *leukocytozoan* (80%) *haemoproteus* (5%), *aegyptianella* (15%). In male chicken, all were infected with *leukocytozoan*. They were all extracellular and they were mostly rounded in shape. Some were microgametocytes and some were macrogametocytes. Some *leukocytozoan* were flagellated.

During the examination of blood smear of Gallus domestic some extracellular elongated gametocytes were observed. Roundish shape poly microgametocytes were observed. The RBC's of the host cell form a narrow dark violet color and also a thin and oval shape with a clear nucleolus. *Aegyptianella* infection was noticed in blood smear of chicken. They were intracellular and extracellular. Some extracellular *Aegyptianella* and some intracellular *Aegyptianella* infection were observed during examination of blood smear of chicken. In our results only the female samples were infected (15%) with *aegyptianella* and no male samples were infected (0%). *Haemoproteus* was intracellular parasite and can be invaginated in the nucleus of erythrocytes. *Haemoproteus* also be observed in our study in the nucleus of erythrocyte of infected blood of *Gallus gallus domesticus*.

Key words Gallus gallus domesticus. Avian blood parasites, Blood smear, Leishman stain, Homoproteus Leukocytozoan

Introduction:

Avian haemoparasites are known to cause high mortality rates, reproductive failures, growth retardation, decreased productivity, and to be pathogenic to domestic poultry. (Samadova, 2017) Several forms of bacteria, bacterial, fungal, and parasitic pathogens can easily infest Gallus gallus domesticus. Chicken is one of the most important domesticated poultry species that is intensively raised and the most advanced and productive enterprise in the development of animals. The industry plays an important role in the supply of animal protein (meat and eggs) to humans. (Opara *et.al.*2014).

Pakistan is a developing country where the poultry sector is a growing industry. In rural economies, it plays a critical role. This field of poultry employs around 5 million people and has a long-term growth trend. It is also an important component of the farming system, giving Pakistan's agricultural people an important role. Chicken can be affected by many health problems, but parasitic infection place a key role. (Nathet *et. al.*2014)

Several recent studies have focused on avian blood parasites as a model system for interactions between host parasites in an evolutionary and ecological sense. (i.e., Bensch et al., 2004; Hell-gren et al., 2004; Ricklefs et al., 2005). A much greater literature exists on these parasites in poultry compared to the reported knowledge on wild birds. Extensive laboratory studies have been carried out describing their pathologies, especially for Leucocytozoon species. (Garnham, 1966; Noblet et al., 1976 Morii, 1992; Nakamura et al., 1997; Ito and Gotanda, 2005). Based on the present taxonomy, 3 Leucocytozoon species and 3 Trypanosoma species are found in domestic Gallus gallus domesticus chicken.

(Sehgal *et. al* 2006) found 3 species of *Leucocytozoon and* 3 species of *Trypanosoma* in the domestic chicken *Gallus gallus domesticus*, mainly in tropical and subtropical regions worldwide. The avian blood parasite causes economic losses and impacts the development of poultry. (Takang*et al.*,2017).

The protozoan disease of birds affecting the blood and tissue cells of the internal organs isleukocytozoonosis. These parasites are assigned to the phylum apicomplexa sub-order haemospororina. Simulidae (black fleas) are used by leukocytozoan animals as vectors. The lifecycle involves reproduction by sporogony in insects with tissue cell schizogony (merogony) and erythrocyte or leukocytes. (Nathet *et al.*,2014). There are two subgenera of the genus Leucocytozoon: Akiba and Leucocytozoon - based on the vector species. Acute outbreaks of leucocytozoonosis in chickens and quails have been identified. Hey, L.caulleryi, L. L., and sabrazesi. Schoutedeni in fowls. Leucocytozoon macleani has been documented in chickens and quails (Valkiunas 1997).

Although the pathogenicity of many Leucocytozoidae (Sporozoa, Haemosporida) species in wild birds is unknown, many cases of mortality in domestic chickens and other poultry specieshave been recorded. (Garnham, 1966; Bennett *et al.*, 1993; Valkiu⁻nas, 2005. In particular, Leucocytozoon caulleryi is virulent; infected chickens also display extreme symptoms of anorexia, ataxia, and anemia and have difficulty breathing.

Haemosporidians are known to be highly pathogenic to domestic poultry with high mortalities. (Hasson 2015). Numerous bird species all over the world can be parasitized by avian haemosporidians. The wide range of infection rates for blood parasites is between 50 percent and100 percent or less. Haemoparasites with significant impacts on avian hosts' physiology, ecology, health, population dynamics, sexual selection and production performance can promote species extinction (Nourani *et al.*, 2018). Haemosporidian parasites are common blood parasites of reptiles, animals, and mammals with some developmental stages in the infected hosts' tissues and circulating blood cells. (Archawaranon, M. 2005). Unicellular eukaryotic genus parasites, Haemoproteus, Leucocytozoon and Plasmoduimim, are the most frequently identified parasites inperipheral blood smears. (Benedikt *et al.*, 2006).

Parasites that affect wild as well as domestic birds (Peirce MA, Bevan BJ.1997). Rickettsialpathogens, which seriously affect most birds, are one of these micro-organisms belonging to the genus Aegyptianella (Tarello and Riccieri 2003, Urquhart *et all.*, 2003). *Aegyptianella* was first described by carpano (1929) in both chickens and geese in Egypt (Castlle MD, Christensen BM.1985.). Within the erythrocytes, *Aegyptianella spp* occurs and the morphology was described by (Thrall MA.*et al.*, 2004). Aegyptianella spp are transmitted by the fowl tick Argas persicus (Gothe R,Hartmann S.1979, Leeflang P, Ilemobade AA.1977).

The presence in the red cells of birds of these rickettsial species can lead to conditions ranging from a stable carrier to a highly pathogenic and sometimes fatal disease. (Castlle MD, Christensen BM.1985, Tarello W.2001).The main clinical sings of acute Aegyptianellosis are fever, anemia, anorexia, diarrhea, pale discoloration of legs, staggering gait and jaundice (Thrall MA.*et al.*, 2004, Tarello W.2001, Soulsby EJL. Helminths.1986)

The goal of our study is to report the presence of different haemoparasites in *Gallus gallus domesticus* in Gulberg town, Karachi Pakistan.

Material and Method:

To study the presence of blood parasites in *Gallus gallus domesticus*, 50 blood samples of broiler chickens 29 females and 21 males were randomly collected from Gulberg Town, Karachi Pakistan during the month February to August 2019 and the ages of the chicken were almost 14- 15 days old. The weight of the chickens was 1.5kg – 2kg.

After the collection of blood sample in anti-coagulant tube, blood smear was prepared on a clean and dry microscopic glass slide by thin smear technique and air dry it. Now, cover the well dried, thin blood smear with undiluted Leishman Stain solution by counting the drops of Leishman stain.Let it stand for 2 minutes, the methanol present in the stain fixes the smear onto the glass slide. After 2 minutes, add twice the amount of distilled water or Phosphate buffer solution and mix the content by swirling or by blowing gently. Rinse the slides thoroughly with Phosphate buffer solution up to 2 minutes or until it acquires a purple-pinkish tinge. Air dry the slides in a tilted position so that the water easily removes out of the slides. Let it dry in air for few hours and then observe the slides under oil immersion objective lens of the microscope with a 100X lens.

Result:

In present study, we examined total 50 blood samples of *Gallus gallus domesticus* where (70%) 35 samples were infected with different blood parasites and (30%) 15 samples were uninfected in which 5 samples of female and 10 samples of males. In 24 samples of female chickens (70%) infection were noticed and in males 11 samples (30%) infection of haemoparasites were noticed. In infected samples of female chicken, we observed 3 different haemoparasites; *Leukocytozoan* (80%) *Haemoproteus* (5%), *Aegyptianella* (15%). In male chicken, all were infected with *leukocytozoan*.(Table 01)

The *leukocytozoan* found are all extracellular and they are mostly rounded in shape. Some are microgametocytes and some are macrogametocytes. Some *leukocytozoan* are flagellated.

Extracellular flagellated *leucocytozoan* is observed in the blood smear of chicken. The exflagellation process of *Leucocytozoon* is a form of sexual maturation, the microgametocyte became underwent a process of maturation with the formation of microgametocyte and subsequent exflagellation. (Fig 1) During the examination of blood smear of *Gallus gallus domesticus* some extracellular elongated gametocytes were observed. (Fig 2). Roundish shape poly microgametocytes were observed between mononuclear erythrocytes, altered the morphology of erythrocytes and made some clusters. (Fig 4). The RBC's of the host cell form a narrow dark violetcolor and also a thin and oval shape with a clear nucleolus. The Rounded macrogametocyte also observed in our study (Fig 3& 5). Another macrogametocyte with some conical end is observed inblood smear of chicken, in this slide morphology of erythrocytes is completely change, some cellscompressed and elongated with compress nucleus. (Fig 6).

Aegyptianella species occur within the erythrocytes. They are intracellular andextracellular. Aegyptianellosis is an acute tickborne febrile disease caused by *aegyptianella species*. Some extracellular *Aegyptianella* are observed during examination of blood smear of chicken (Fig7) and some intracellular *Aegyptianella* infection were observed in blood smear of chicken (Fig 8). In our results only the female samples were infected (15%) with *Aegyptianella* and no male samples were infected (0%). *Haemoproteus* is intracellular parasite and can be invaginated in the nucleus of erythrocytes. *Haemoproteus* can be observed in the nucleus of erythrocyte of infected blood of *Gallus gallus domesticus* (Fig 9).



Figure 1: Blood smear of chicken (Gallus gallus domesticus) infected by Exflagellatedleucocytozoon sp.(100X)



Figure 2: Image of blood smear of Gallus gallus domsticus infected withleucocytozoon spp. Elongated gametocytes.

(100X).



Figure3: Blood smear of chicken infected with round macrogametocyte of *leukocytozoan* spp. (100X)



Figure4: Microgametocyte of *leukocytozoan* from blood smear of chicken (round gametocytes) (100X).



Figure 5: Blood smear of chicken showing infection of roundish shape macrogametocytes of *leukocytozoan* spp.



Figure 6: Blood smear of chicken infected by *leukocytozoan* (rounded macrogametocyte) (100X).



Figure 7: Extra cellular Aegyptianella spp. in bood smear of infected Gallus gallusdomesticus. (100X)



Figure 8: Intracellular Aegyptianella specie in different blood smears of Gallus gallus domesticus





Figure 9: Intracellular parasite Heamoproteus spp. observed in infected blood sample of Gallus gallusdomesticus.

Prevalence in Chicken Blood Smear		
Parasite specie	Male	Female
Leucocytozoan	100%	80%
Heamoproteus		5%
Aegyptianella	_	15%

TABLE # 1: Showing prevalence of infection in blood of Gallus gallus domesticus.



Piechart showing prevalence of infection in Gallus gallus domesticus

Discussion:

In present study we detected round and oval gametocytes of *leukocytozoan* and macro and microgametocytes of *Leukocytozoan*. There were numbers of reports on *Leucocytozoan* in many birds in Africa (Huchzermeyer.FW.1973,Permin.A.*et.al.*, 2002), Israel (Gill H.*et.al.*, 2005), New Zealand , Spain (Merino S.*et al.*, 1997), and USA (Stuht JN.*et.al.*, 1999). However, this is the firststudy the prevalence and ultrastructural investigation of *Leucocytozoan* among *Gallus gallus domesticus* in Gulburg town. The current data demonstrated that *Leucocytozoan spp.* infections are distributed in the study area chick population, These findings are agree with (Dezfoulian *et al.*, 2013), Operated in Southwestern Iran on Leucocytozoanosis in Domestic Birds: An Ultrastructural Analysis in Lorestan Province. Leukocytozoon species have infected a total of 44 (16.0 percent)

birds. In these results, birds with relatively high Leukocytozoan prevalence included chicken with 5.1% (n=14) infected, geese with 4.3% (n=12) infected, ducks with 3.6% (n=10) and turkeys with 2.9% (n=8) infected.mostly chick are infected with *leukocytozoan*. Haemaprotozoa infection has been suggested to be species-specific; host range and gametocyte characteristics, such as staining characteristics, size, nature, and degree of host cell distortion and altered shape, and host cell nucleus location, are criteria for species designation. (Greiner, E.C.*et al.*, 1994, Forrester, D.J.*et al.*,2001, Remple, J.D.2004)

In present study we found round and elongate form of *Leucocytozoon* gametocyte. Ahmadov *et al.* (2019) completely agree with the present study. An elongate form and round formwere observed in birds. The appearance of both round and elongated gametocytes evidenced that the *Leucocytozoon* is a pathogenic strain. They found only the elongated form of the blood parasite in chickens. Though both elongated and round forms were observed but there were more elongated forms. In recent study, exflagellated *leucocytozoan* is observed in blood smear of chicken. Garnham (1967) observed Exflagellation in *Plasmodium*, Hepatocystis and *Leucocytozoan*. Exflagellation of mature microgametocytes took a place in a moist environment within 2.5 to 3 minutes. In present study we observed exflagellated *leucocytozoan* in blood smear of chicken (Fig1). The factors including surface tension, temperature change, mechanical force and pH werelisted as being responsible for the rupture of host cell membrane (Dezfoulian *et al.*, 2013).

Ozmen *et.al.*, (2009) examined roundish and fusiform gametocytes in the red blood cells. Gametocytes in roundish shape was rarer than fusiform in host cell in the blood smear of buzzard (Buteo buteo). These findings agree with the present study and roundish microgametocytes observed in blood smear of *Gallus gallus domesticus*(Fig 4).

In recent study, macrogametocyte of *Leucocytozoon spp.* Observed from blood smears of chickens (Fig5). These findings were also observed by Samadova *et al.*, (2017). They observed themature round gametocytes of *Leucocytozoon* sp. from chickens in erythrocytes. The measurements of the macrogametocyte of *Leucocytozoon* spp. from blood smears of chickens were $14.6\pm0.3\mu$ M×12.7 $\pm0.19\mu$ M. Invasion intensity ranged from 1-2 per 100 microscopic fields. The nucleus of parasite was $3.75\pm0.13\mu$ M×3.0 $\pm0.09\mu$ M in measurement. In spindle-shaped host cellsthe macrogametocyte of *Leucocytozoan* sp. of quails was $13.4\pm0.15\mu$ M×5.0 $\pm0.11\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in measurement. The complete round gametocytes of *Leucocytozoon* sp. of quails was $13.4\pm0.15\mu$ M×5.0 $\pm0.11\mu$ M in measurement of *Leucocytozoon* sp. of quails was $13.4\pm0.15\mu$ M×5.0 $\pm0.11\mu$ M in measurement. The nucleus of parasite was $13.4\pm0.15\mu$ M×5.0 $\pm0.11\mu$ M in measurement. The macrogametocyte of *Leucocytozoon* sp. of quails was $13.4\pm0.15\mu$ M×5.0 $\pm0.11\mu$ M in measurement. The nucleus of parasite was $13.4\pm0.15\mu$ M×5.0 $\pm0.11\mu$ M in measurement. The nucleus of parasite was $13.4\pm0.15\mu$ M×5.0 $\pm0.11\mu$ M in measurement. The nucleus of parasite was $13.4\pm0.15\mu$ M×5.0 $\pm0.11\mu$ M in measurement. The nucleus of parasite was $13.4\pm0.15\mu$ M×5.0 $\pm0.01\mu$ M in measurement. The nucleus of parasite was $13.4\pm0.15\mu$ M×5.0 $\pm0.01\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in measurement. The nucleus of parasite was $2.1\pm0.1\mu$ M×1.5 $\pm0.08\mu$ M in

In the current research, haemoproteus was found at a prevalence rate of 5 percent, while Haemoproteus spp. was found by Hasson (2017). With a prevailing rate of 6 (17.6 percent). The variations identified in the prevalence of avian haemoparasite infection are due to variables associated with the methods used for diagnosis, breeding season, sampling effort and location, including poultry species and the richness of arthropods. vectors responsible for transmission the parasites (Braga et al., 2011;Gimba et al., 2014). Occurrence of Haemoproteus spp. detected by Nourani (2018)(Haemosporida: Haemoproteidae) from the New Host Records of East Iranian Passerine Birds. Rate of infection by age group for Haemoproteus spp. In contrast with immature hosts, a higher infection rate for mature people (39.13 percent) was shown (28.57 percent). Yoshimura et al. 2014 observed that the haemosporidian infection rate in Japan's Columbiformes, Anseriformes and crow species (Passeriformes) was 21%, 17% and 93.8%, respectively. Prevalence is a dynamic parasite parameter and appears to be affected by a large number of variables, such as age, gender, habitat, altitude, immune system, etc.

In present study rate of infection of *Aegyptianella spp*. was 15% out of 50 chicken was detected. (Suleiman 2012) observed 50 chicken for *aegyptianella spp* infection and found (20%) infection in chicken. Rate of frequency can be differ because of many factors like age, environment, immunity etc.

Al-Alousi *et al.* (1994) reported 2% infection rate with *Aegyptianella spp.* among this study of endoparasites in turkeys in Mosul city. Desfoulian *et al* (2011) observed that in chickens, ducks, geese and turkeys, the percentage of infection was 33.3 percent, 9.5 percent, 33.3 percent, 23.9 percent, and the overall percentage of infection was 7.6 percent. Swai (2011) found that Aegyptianella is highly pathogenic to birds, especially chickens, with a 30-80% mortality risk among young birds, and Aegyptianella is identified as one of the major hemoparasites in the production of poultry. Poulsen (2010) observed that Aegyptianella infection is prevalent among free-range birds raised in the tropics, with a prevalence of 9% and 6% in Ghana and Zimbabwe, respectively. Dezfoulian 2011 observed the Aegyptianella spp. prevalence. In the northern, southern and central regions of the province of Lorestan, four genera of domestic poultry have been evaluated. Out of 275 birds used in this study, 21 (7.6 percent) were contaminated with the species. Seven chickens (33.3 percent), 2 ducks (9.5 percent), 7 geese (33.3 percent), and 5 turkeys (23.9 percent), respectively, were detected with Aegyptianella.

Conclusion

In conclusion, the study of blood parasites in Gallus gallus domesticus in Gulberg town, Karachi, revealed the presence of various blood parasites in avian blood. Out of the 50 blood samples of Gallus gallus domesticus examined, 35

samples were found to be infected with different blood parasites, while 15 samples remained uninfected. Among the infected samples, the prevalence of blood parasites was higher in female chickens (70%) compared to males (30%).

The most common blood parasite observed in infected female chickens was leukocytozoan, accounting for 80% of infections. Haemoproteus was observed in a smaller proportion (5%), and aegyptianella accounted for 15% of infections in female chickens. On the other hand, all infected male chickens showed the presence of leukocytozoan. This study shed light on the prevalence and diversity of blood parasites in Gallus gallus domesticus in Gulberg town, Karachi. The findings suggest that female chickens were more susceptible to certain blood parasites, such as aegyptianella, while leukocytozoan was prevalent in both male and female chickens.Understanding the presence and characteristics of these blood parasites is crucial for the management and control of avian diseases in the region. Further research and monitoring are warranted to gain a comprehensive understanding of the impact of these blood parasites on the health and well-being of Gallus gallus domesticus in the area.

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