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# Preliminary investigation of hematozoan in laughing Dove (*Spilopelia senegalensis*) in Suleimani Province

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## Abstract

*Spilopelia senegalensis*, a species of laughing dove native to Iraq, has successfully adapted to the Kurdistan Region. The world's sub-Saharan Africa, Arabia, Iran, Iraq, Afghanistan, Pakistan, India and some other Middle East countries were among the regions it settled. The laughing dove first appeared in the middle and southern regions of Iraq in 2005. In the current study, 100 laughing doves (*Spilopelia senegalensis*), of various ages and sexes, were examined microscopically using blood smears stained with Giemsa. Fecal samples were aseptically taken from the intestines after the birds had been slaughtered and fully bled. Both centrifugal sedimentation and flotation techniques were used to analyze the fecal samples. The results of the investigation showed that 43 out of 100 doves (43% of them) had a plasmodium species infection. We documented that the most infective parasite infection in doves and this will be the first report in Sulaimani Province, Kurdistan Region, Iraq.

**Keywords:** Parasite, *Spilopelia senegalensis*, Plasmodium, Capillaria.

## Introduction

Laughing Dove, *S. senegalensis* (Old Name: Little Brown Dove) The laughing turtle dove and the palm dove are other names for this bird. The Columbidae family, genus Spilopelia, includes the Laughing Dove (1). The world's sub-Saharan Africa, Arabia, Iran, Iraq, Afghanistan, Pakistan, and India (2) were among the regions it settled. The laughing dove first appeared in the middle and southern regions of Iraq in 2005, as opposed to the north of Iraq, where it was first observed in 1970. It stood out thanks to its short stature, leaner body, and reddish-brown feathers. (3). Small seeds that fall from grass, grains, cereals, fruits, berries, nectar, and other vegetable partsare all food sources for *S. senegalensis*. Small ground insects like termites and beetles are their primary food sources (4). The laughing dove got its name from its distinctive coo, which sounds similar to human laughter (5).

Birds' respiratory systems, which include air sacs and syrinx organs that are related to flight and voice production, are more specialized than mammalian respiratory systems (6). The nasal cavity, larynx, trachea, syrinx, bronchi, lungs, and air sacs make up the respiratory system in birds, whereas the nasal cavity, larynx, trachea, bronchi, and lungs are all present in mammals (6, 7). Many different parasitic, bacterial, viral, and fungal infections can affect avian species. Among the many parasitic diseases that affect birds, hemoparasitic infections are now understood to be of the utmost importance (8). Numerous bird species harbor blood endoparasites called haemosporidians (Sporozoa: Haemosporida) (9). Blood parasites are extremely important economically because of things like declining mortality rates (10), restricted growth, and the inability to reproduce (11). A series of events known as a diminished immune response take place when immune cells, including white blood cells, are destroyed (12). The identified species of avian blood protozoon parasites number more than 200 (13). The most common avian hemoprotozoan species are *Leucocytozoon, Plasmodium, Haemoproteus, Aegyptinella, Eperythrozoon, Fallisia, Haemobartonella*, and Trypanosomes. (14).

Blood parasites display various developmental stages in the tissues and circulating red blood cells (RBCs) of the infected hosts. Both domesticated and wild birds can become infected with that intracellular hemosporidian (15, 16, 17). Although they have some host-specificity, *Haemoproteus* and *Leucocytozoon spp*. are only found in bird species that are members of the same family. Plasmodium species have the ability to infect many different bird families by changing their genetic makeup and physical characteristics (18). According to studies (19, 20), mosquitoes, *Simulium species*, midges or hippoboscid flies, *Argas persicus*, and *Culex species*, respectively, transmit avian Plasmodium, *Leucocytozoon, Haemoproteus, Aegyptianella*, and *Trypanosome spp.* The behavior of the birds may be adversely affected by hemoparasites, which can also cause depression, anorexia,

decreased productivity, growth retardation, green feces, and death (21).

The parasitic disease known as "avian malaria" is brought on by protozoan parasites of the *Plasmodium genus* (22). More than 1,000 different avian hosts have been found to harbor over 65 different Plasmodium species (23). Hemolytic and severe anemia, a high number of immature erythrocytes, pale watery blood, lymphocytosis, leukocytosis, hypoalbuminemia, diffuse patches of extramedullary erythropoiesis in the liver and kidney, and hemoglobinuria are all symptoms of the malaria parasite Plasmodium (24, 25). Blood parasites have been identified using conventional microscopic examination of stained blood smears and/or molecular techniques utilizing polymerase chain reaction (PCR) assays (26, 27). Finding out the most common blood parasites that infect the dove in the Sulaimani region is the main goal of this investigation.

## Materials and Methods

**Animals of the Study:** A total of 100 seemingly healthy laughing doves (*S. senegalensis*) of various ages were caught alive at random in Sulaymaniyah Province/Kurdistan Region, Northern Iraq, and transferred to the Clinical Pathology Laboratory in the Department of Clinic and Internal Medicine, College of Veterinary Medicine, University of Sulaimani. They were then marked with serially numbered bands to prevent repeated sampling.

**Figure 1: laughing Dove (*Spilopelia senegalensis*)**

**Sampling**

**Blood Sample:** Approximately 1.0 mL of blood was drawn for each sample provided with two blood smears for morphologic identification of haemoparasites after the wing vein (brachial vein) was cleaned with 70% ethyl alcohol. Within 5 to 10 minutes, blood films were air-dried, after which they were fixed in absolute methanol for 5 minutes, stained for 50 minutes with Giemsa (3%) working solution, and finally the excess stain was removed with buffered water before the films were allowed to dry. Blood parasites were checked for in blood films using a 100-percent oil immersion method.

**Fecal Sample:** One hundred birds were used in this study and they were humanely sacrificed by slaughtering within 2 hours of collection and allowed for complete bleeding. The feathers were removed and the birds were dissected aseptically from the abdominal site. Then, fecal samples were aseptically taken from the intestine, approximately, 2-5g of feces from each bird. This study was approved by the ethical committee of the College of Veterinary Medicine, University of Sulaimani.

**Parasitological examination**

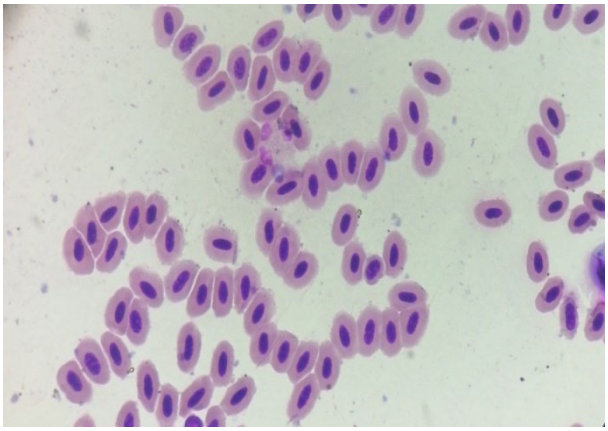
**Analysis of fecal samples:** For fecal examination, both centrifugal sedimentation technique and floatation technique were used as per procedure recommended.

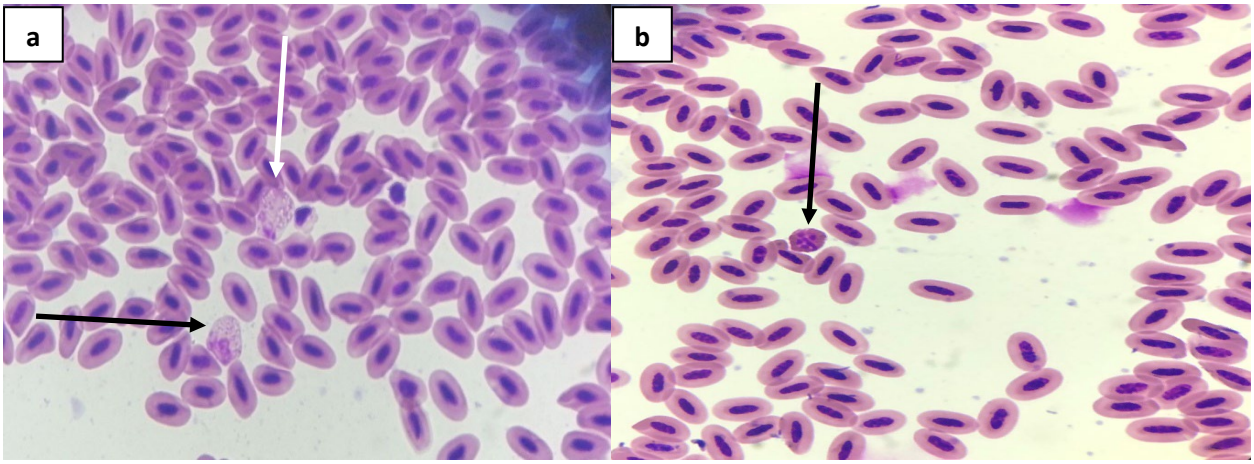
**Centrifugal sedimentation technique:** Fecal samples were collected and placed in a 100 ml beaker before being completely emulsified with 10 to 15 ml of water. The contents of a cup were filtered using a strainer and then funneled into centrifuge tubes. The centrifuge tubes were balanced before being centrifuged at 5000 rpm for 5 minutes. Using Pasteur pipettes and a bulb, a small portion of the top layer of sediments was transferred to a clean microscopic slide. Following that, the cover slips were placed over the sediment drop, and the slide was examined with both low and high-power objectives.

**Floatation technique:** Fecal samples were collected and thoroughly emulsified with 10 to 15 ml of saturated sugar solution in a 100 ml beaker. The mixture was poured into a cup after being strained through a strainer, and it was then transferred into a floatation tube until it reached the tube's brim and formed a positive meniscus. The mixture was then left undisturbed for 15 to 20 minutes. The tip of the positive meniscus was gently touched with a clean cover slip, which was then placed on a slide and examined under a microscope using both low and high-power objectives.

## Results

A total of 200 thin blood smears from 100 doves (two blood smears per bird) were examined; 43 (43%) were found to be Plasmodium spp. infected, while only two (2% of the fecal samples) were infected by a gastrointestinal parasite (*Capillaria spp*.) (Fig. 3). In the current study, none of the infected birds had displayed any ill-effects clinically. When male and female gametocytes, schizonts, and the ring stage were found in the blood smear, Plasmodium spp. was recognized (Fig. 2).




a
b


c

**Figure 2: a. Female gametocyte (black arrows) and Male gametocyte (white arrow), b. Schizonts (black arrows), and c. Ring stage (black arrow)**

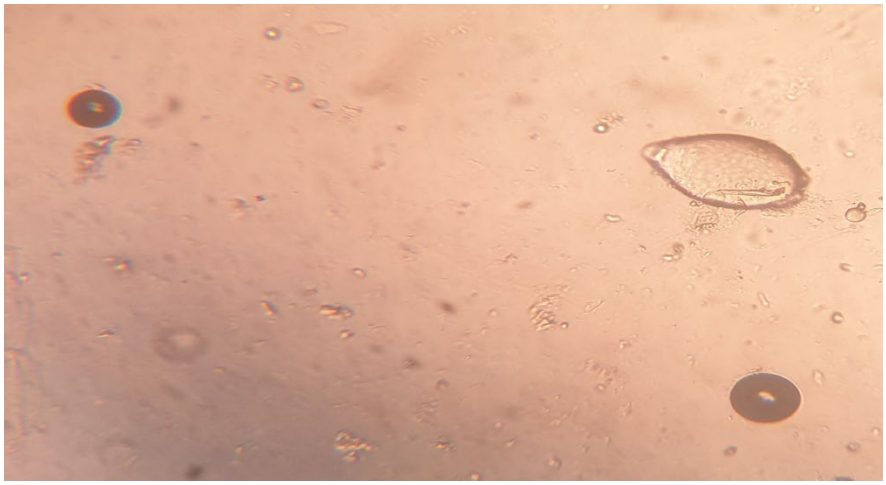


**Figure 3: Egg of *Capillaria spp***

## Discussion

The Laughing Dove *S. Senegalensis* is a Columbiform native to most of sub-Saharan Africa, Middle-East and the Indian subcontinent (28). Following Giemsa-stained blood or tissue smears, the various haemoparasite developmental phases could be identified under a light microscope. The molecular detection methods of nucleic acid amplified and sequenced are beneficial, effective, and sensitive in identifying the various stages of parasites in the blood or tissues. Even in cases when blood smears are negative (29). Although several studies have demonstrated that, the sensitivity of molecular and microscopic identifications for finding avian hemoparasites is equivalent (30, 31). In the evolutionary tree of *Plasmodium* and similar Haemoprotozoon, mitochondrial genes (mtDNA) and genomic markers have been constructed and are usually utilized in the molecular-based identification of avian blood parasites (32).

Contrary to a study conducted in Garmian administration, Iraq, which found no infections with Plasmodium, Leucocytozoon, or other blood parasites and only the protozoan parasite Haemoproteus columbae in doves and domestic pigeons, the current study discovered that 43% of the examined doves were *Plasmodium species* infected (33). The variation in prevalence rates may be brought on by various management strategies and an abundance of

arthropod vectors capable of transmitting the parasite.

The study was conducted between January and October, and because it offers favorable climatic conditions (including temperature and humidity), the rainy season is typically the best time of year for hatching major arthropod vectors, such as mosquitoes and other flies. This conclusion is supported by the fact that trypanosomiasis and malaria, two blood parasite diseases that are spread by arthropods (such as mosquitoes and ticks), are more common in both human and animal populations during the rainy season (34). Furthermore, due to overgrown plants and vegetation brought on by agricultural farming activities, ponds of water from inadequate drainage systems, and the ideal humidity and temperature needed for egg hatching, increased breeding of arthropod vectors, such as mosquitoes, may occur during the rainy season. For arthropod reproduction, on the other hand, the hot, dry season is typically not regarded as either desirable or advantageous because the heat has the potential to cause egg death and kills arthropod larva. The findings of this investigation are in line with those of (35), who noted that in Bangladesh, during the rainy season, there was a higher incidence of avian malaria parasites. Additionally, according to (36) the infection rate in Iran was highest in the autumn (44%) and lowest in the spring. It is obvious that avian hemoparasites can put bird populations in danger and may have an effect on how long their insect vectors persist.

## Conclusion

Blood parasites known as *Plasmodium* species are widely dispersed and have been found in numerous bird families. Climate variables like temperature, humidity, and vector activity have a big impact on how infections spread. Monitoring the prevalence of avian hemoparasites in domestic poultry and wild migratory birds is necessary to reduce the risks of potential outbreaks. Microscopical examination of thin blood smears stained with Giemsa is the most reliable and cost-effective method for detecting blood parasites. To quickly identify the haemosporidian parasite species and understand the ecological and evolutionary interactions among hosts, parasites, and vectors in this area, it is advised to conduct additional research using molecular diagnostic techniques. It would be advantageous for scientists to carry out a thorough investigation of the distribution of vector communities and how they relate to hematozoa in these regions, as this could reveal how specific hematozoan lineages are distributed regionally.

## Conflicts of interest

The authors declare that there is no conflict of interest

## Ethical Clearance

This work is approved by The Research Ethical Committee

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**تقصي أولي عن الهيماتوزوان في الحمام الضاحك (*Spilopelia senegalensis*) في محافظة السليمانية**

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## الخلاصة

*Spilopelia senegalensis*هو نوع من الحمام الضاحك موطنه العراق، نجح في التكيف مع إقليم كردستان. وكانت أفريقيا جنوب الصحراء الكبرى في العالم والجزيرة العربية وإيران والعراق وأفغانستان وباكستان والهند وبعض دول الشرق الأوسط الأخرى من بين المناطق التي استقر فيها. ظهرت الحمامة الضاحكة لأول مرة في المناطق الوسطى والجنوبية من العراق عام 2005. وفي الدراسة الحالية، تم فحص 100 حمامة ضاحكة(*Spilopelia senegalensis)* ، من مختلف الأعمار والأجناس، مجهريا باستخدام مسحات الدم الملطخة بالغيمزا. تم أخذ عينات من البراز بطريقة معقمة من الأمعاء بعد ذبح الطيور ونزفها بالكامل. تم استخدام تقنيات الترسيب والتعويم بالطرد المركزي لتحليل عينات البراز. وأظهرت نتائج التحقيق أن 43 من أصل 100 حمامة (43٪ منها) أصيبت بعدوى البلازموديوم. قمنا بتوثيق أن أكثر حالات العدوى الطفيلية في الحمام سيكون هذا التقرير الأول في محافظة السليمانية، إقليم كردستان، العراق.

**الكلمات المفتاحية:** طفيل، *Spilopelia senegalensis*، *Plasmodium, Capillaria.*