A Comparative Study of Blood Parasites Naturally Occurring in Doves and Domestic Pigeons in Garmian Area-Iraqi Kurdistan Region

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Abstract

The aim of this study was to compare the infection rate of the blood parasites naturally occurring in doves and domestic pigeons in Garmian area- Iraqi Kurdistan region during the period of 1st December 2015 to 1st May 2016. A total of 234 apparently healthy doves [129 (56 males and 73 females)] and domestic pigeons [105 (59 males and 46 females)] of different ages and sexes were randomly examined microscopically using Giemsa stained blood smears. The results of the study indicated that *Haemoproteus columbae* was the only parasite that had been detected. Out of 129 total numbers of examined doves, 14 doves were found to be infected at an infection rate of 10.85% [7 (12.50%) males and 7 (9.58%) females] and 11 pigeons out of 105 total numbers of examined domestic pigeons was found to be infected with *H. columbae* at an infection rate of 10.48% [6 (10.17%) males and 5 (10.87%) females], the infection rates of this haemoparasite for both bird species were non-significant (P>0.05). It was concluded from this study that the doves and domestic pigeons are susceptible to *H. columbae* and the rate of occurring in both bird species is somewhat the same. The current study was conducted for the first time in this area and revealed the existence of *H. columbae* protozoan.

Keywords: Blood parasites, comparative study, domestic pigeons, doves, Garmian area-Iraqi Kurdistan region

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دراسة مقارنة لطفيليات الدم التي تحدث بشكل طبيعي في الفختة والحمام المنزلي في منطقة گرميان في إقليم كردستان – العراق

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الخلاصة

إن الهدف من هذه الدراسة هو مقارنة معدل الإصابة بطفيليات الدم التي تحدث بشكل طبيعي في الفختة والحمام المنزلي في منطقة گرميان اقليم كردستان – العراق خلال الفترة من الأول من كانون الأول 2015 إلى الأول من أيار 2016. من إجمالي 234 فختة والحمام المنزلي من مختلف الأعمار ومن كلا الجنسين الذين كانوا أصحاء ظاهريا منها 129 فختة (56 ذكور و73 إناث) و105 حمام منزلي (59 ذكور و46 إناث) وفحصت العينات الدموية المصبوغة بصبغة الغيمزا مجهريا وبشكل عشوائي. من أصل 129 من الفختة الذين تم فحصهم، وجدت 14 منها مصابة بنسبة 10.85% ومن ضمنها 7 (12.50%) من الذكور و7 (9.58%) إناث، حيث وجد أن 11 حمام من مجموع 105 مصابين بالطفيلي H. columbae بنسبة 10.48% منها 6 (10.17%) من الذكور و5 (10.87%) من الإناث، حيث كانت نسب الإصابة بهذه الطفيليات لكل من الفختة والحمام المنزلي غير معنويا (P> 0.05) .نستنتج من هذه الدراسة بأن الفختة والحمام المنزلي عرضة للإصابة بالطفيليH. columbae بنسبة إصابة متساوية. وقد أجريت هذه الدراسة الحالية للمرة الأولى في هذا المجال في منطقة گرميان.

الكلمات المفتاحية: طفيليات الدم، دراسة مقارنة، الحمام المنزلي، الفختة، منطقة گرميان اقليم كردستان– العراق

Introduction

Haemosporidian parasites are vector-born parasites in the order Haemosporidia (Phylum: Apicomplexa) that are commonly found in reptiles, birds and mammals, avian haemosporidian parasites are taxonomically diverse and cosmopolitan in distribution, especially in warm and temperate climate (1, 2, 3) also they are intracellular protozoa found within the blood cells and tissues of their avian hosts belonging to the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*, these parasites are transmitted to susceptible birds by insect vectors, which include mosquitoes, black flies, sand flies, biting midges (Culicoides), and louse flies (Hippoboscidae) (2, 4, 5). The infective stage is the sporozoite which is present in the salivary glands of the insect vector (6). The genus *Haemoproteus* includes a large number of intracellular protozoan parasites of birds distributed all over the world (7), asexual development of this parasite occurs in the peripheral blood of the birds and sexual development in the vector louse fly (5). The gamonts of *H. columbae* develop from tiny forms to elongated, crescent-shaped forms, which partially surround the nucleus of the host red blood cells (8). *Haemoproteus columbae* widely occurs in doves and pigeons in tropical and subtropical regions (9, 10). It is usually non-pathogenic and only causes a disease when the birds are stressed, The pathogenic manifestation of avian haemoparasites has been associated with mortalities, reproductive failure, retardation of growth, reduced productivity and may exert negative effects on behavior and community structure (11). Factors extrinsic to hosts, such as geographical region (12) and time of season or year (13) are important because they can influence the distribution and abundance of infection stages or vectors. however, geographic location and genetic are the most important factors that determine resistance to bird haemosporidians, although the age of the bird, strain of the parasite, and stress may also play roles in the pathogenesis of blood parasites (14). Many studies have been recorded on avian blood parasites in different areas of the world (15, 16, 17). Numerous haemoparasites have been associated with avian species both in the wild and the domestic birds, and it is estimated that 68% of all bird species are susceptible to haemosporidians (18). Doves and domestic pigeons (family: Columbidae) are ubiquitous and can be found in virtually every town and city around the globe (19, 20), feed on wide range of food items, which include grains, slugs, earthworms, and insects (19, 21), they have been used for a long time as a meat resource, laboratory animals, pets or cultural and religious symbols. Also the role of these birds in the transfer of diseases to humans and domestic animals, especially in connection with intensive poultry production, has been well documented. Birds may be infected with many species of parasites, including *Haemosporidia* of the genera *Plasmodium*, *Haemoproteus*, *Haemogregarinidae*, *Leucocytozoon* of the genus *Hepatozoon* and *piroplasmids* of the genus *Babesia* (22), some of which are pathogenic to humans (21, 23). Studies, to date, have determined that the most common blood parasite found in columbids is *Haemoproteus* and infection rate may be as high as 75% ranging from 6 to 86% (24, 25, 26, 27, 28, 29). Haemoparasites in these bird species have been studied in several countries (30, 31).Haematozoa of Iraqi birds were documented for the first time and the knowledge were given on 77 species of birds (32) and after that in Mosul area *Haemoproteus columbae* in rock dove was casually reported (33). Between the years 1990 to 2004 a series of 9 papers had been published surveying blood parasites of nearly 1/4th of the total number of avian species of Iraq (34, 35, 36, 37, 38, 39, 40, 41). The present investigation originally designed and conducted to provide data on comparative infection rates of blood parasites naturally occurring in doves and domestic pigeons between1st December 2015 to 1st May 2016 in different localities of the Garmian area (Garmian administration)-Iraqi Kurdistan region. It is visualized that the

data will assist in identifying the host range and contribute to a long term database on the occurrence of this pathogen among wild and domestic avifauna in the country.

Materials and Methods

Doves and domestic pigeons involved in this study were examined between1st December of 2015 to 1st May of 2016 in different localities of the Garmian area (Garmian administration)-Iraqi Kurdistan Region, its capital is Kalar district, which is geographically located at150 Km. South east of Sulaimani district center. A total of 234 apparently healthy doves [129 (56 males and 73 females)] and domestic pigeons [105 (59 males and 46 females)] of different ages and sexes were randomly captured alive, kept in cages, and marked with serially numbered bands to avoid multiple sampling (Fig. 1). After the wing vein (brachial vein) was cleansed with 70% ethyl alcohol, approximately 1 ml of blood was collected for each sample provided with two blood smears for morphologic identification of haemoparasites. The smears were air-dried, fixed in absolute methanol for about 5 minutes, and then were stained with Giemsa 3% working solution from stock Giemsa's stain for 50 minutes, then the excess stain was washed off with buffered water and left to dry, blood smears were carefully examined microscopically under an oil immersion lens (100X) for haemosporidians, gametocytes of *Haemoproteus columbae* were photographed (42). The parasite was identified by detection of intra erythrocytic gametocyte stage morphologically (9, 43, 44). Chi square test analysis was used to compare infection of the parasite between the doves and domestic pigeons as well as their sexes using SPSS programme. The differences were considered significant at $P<0.05$. Photos were taken with iPhone 6 (s) model MKQR2AH/A, camera12- megapixel.

Fig. (1): Showing A- Doves, B-Domestic pigeons.

Result

Two hundred and thirty four apparently healthy doves [129 (56 males and 73 females)] and domestic pigeons [105 (59 males and 46 females)] of different ages and sexes were used in this study, from which the only encounterd haemoparasite in both the doves and domestic pigeons on microscopic examinations of the Giemsa-stained blood smears of this study was gametocytes of *Haemoproteus columbae* which were seen inside red blood cells (Fig.2). Out of the total examined birds (234), 25 were infected with an overall infectionrate of 10.68%, separately the infection rates were 10.85% and 10.48% in doves and domestic pigeons respectively, comparatively the results of the study showed that the infection rate with *H. columbae* was little higher in doves (10.85%) than in domestic pigeons (10.476%) with non-significant difference (χ^2= 0.09, P=0.926 Table-1), as general, in regard to the sexes, male bird infections (11.30%) was higher than the females (10.08%) with no significant difference statistically (χ^2 = 0.091, P=0.763 Table-1), moreover the infection rates of parasitemia

with current parasite was some higher in male doves (12.50%) than that in male domestic pigeons (10.17%), while the rate in female doves (9.58%) was lower than that recorded in female domestic pigeons (10.87%), comprehensive data is shown in Table-1. The result of the Giemsa stained blood smears of examined birds infected with *Haemoproteus* parasites revealed the presence of intraerythrocytic gametocytes (macrogametes and microgametocytes) occupying about one-half of the erythrocyte cytoplasm with little or no displacement of the host cell nuclei; they surround the nuclei either partially forming a "halter-shaped" appearance or completely (Fig. 2). It is noteworthy that there is no internal and external pathological changes associated with theinfection of this parasite in both doves and domestic pigeons were grossly observed along the period of the examination.

Table (1) Infection of *Haemoproteus columbae* infection in doves and domestic pigeons in Garmian area-Iraqi kurdistan region (N=234)

Birds	No. of examined birds		No.and % of infected birds		Infection
	Male	Female	Male	Female	
Doves (129)	56	73	7 (12.5%)	7 (9.58%)	14 (10.85%)b
Domestic pigeons (105)	59	46	6 (10.17%)	5 (10.87%)	11 (10.48%) **b**
Total % (234)	**115**	**119**	**13 (11.3%) a**	**12 (10.08%) a**	**25 (10.683%)**

Values with same superscripts within a row and column were non-significant (P>0.05).

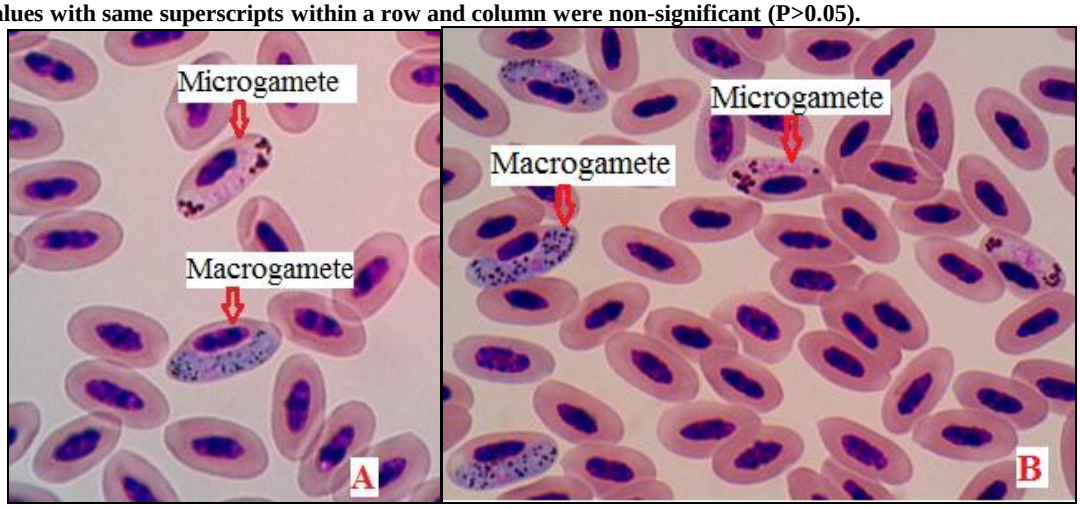

Fig. (2) Macrogametocyte and microgametocytes of *Haemoproteus columbae* (1000X) within red blood cells in doves (A) and domestic pigeons (B) stained with Giemsa's stain.

Discussion

Haemosporidians are one of the most well-known and well-studied group of parasitic protists because they include the causative agents of human malaria (2). Till now, studies in Kurdistan Region- Iraq, trailed to elucidate the infection of blood protozoa in chickens and turkeys, being 17.36 % and 19 %, respectively (8, 45) but no attempt was carried out to investigate the presence of blood protozoa in columbid birds, and this was the objective of the study. The results of this study provide an indication of the infection of blood parasites in doves and domestic pigeons of the family Columbidae found in Garmian administration, Kurdistan Region-Iraq. In the present study only *Haemoproteus columbae* protozoan parasite was identified in both bird species, as well as no any infections with *Plasmodium*, *Leucocytozoon* or other blood parasites were observed that might be due to the seasonal variation or absence of vectors, which play an important role in the transmission of infection (45). Out of the total examined birds (234) in our study, 25 were infected with an overall infection of 10.68% (11.30% male, 10.08% female). Which is relatively low compared to a study

carried out in Iraq where an overall infection rate was 60% for rock pigeons (*Columbia livia*) (31). Statistically, in the current study no significant difference in the infection rate between doves (10.85%) and domestic pigeons (10.48%) and as well as between males and females in both bird species separately, this was corresponding by (46) they found non-significant difference in sex for the infection of *Haemoproteus*. Therefore, the absence of statistical significant difference of infection between both bird species and identifying the same parasite (*H. columbae*) in them may be indicated that the both birds have almost the same body resistance against the blood parasites infections. However, *Haemoproteus* spp. protozoa are widely distributed in tropical and subtropical regions (10) and the overall highest infectivity of *H. columbae* parasites was recorded during the summer season (30), and this may be due to the high level of blood parasite vectors that available at the hot seasons, so these above statements support the low infection load of *H.columbae* in current study which was done almost in the wet seasons (winter and most of spring) (47, 48). Because parasite infection largely depends on exposure to vectors, the absence of sex-related differences in this infection suggests that the sexes were equally exposed to vectors. This agrees with the fact that, in columbid birds of both sexes live in the habitat and participate in the breeding duties in a similar fashion (49). The result of the current study was approach to the findings of researchers such as (50) in Pakistan who found that 12.20% of pigeons were infected with *H. columbae,* (42) in Nigeria who recorded that 11.60% of wild pigeons and doves harbored *Haemoproteus* sp., also (51) in Nigeria, it was reported that 14% of pigeons (*Columbia livia*) infected with *Haemoproteus* species. In contrary, previous studies regarding the identified *Haemoproteus* infection conducted in rock pigeons in Iraq (31), in domestic pigeons at South Khorasan- Iran (52), in wild pigeons in Iran (53), in wild pigeons in Ankara District (54) revealed high infection rates of 60%, 47.05%, 50%, 57% respectively. While studies (55, 56, 57) were done in Iran which almost have the lower overall infection rate they were reported 24%, 30% *and* 17.47% pigeons are positive for *Haemoproteus columbae* respectively. Our results agree with the conclusion of (58)they referred to the reduced presence of parasite vectors in urban areas as a reason for the low parasitemia in urban birds, this may be back to that urban areas may provide increased food and water resources, which can enhance immune capacity to resist infection and the ability to eliminate parasites. Resistance to blood parasites in birds may be genetic or may be more prevalent in birds from certain locations (59). Also resistance may be associated with age, as older birds may have an acquired immunity (60, 61). In the present study, the examined birds exhibited low infection load (10.68%) of *H. columbae* compared to previous studies conducted in Iraq and world. This low infection could be attributed to the presence of scarce number of blood sucking vector flies during the wet seasons (December, January, February, March and April) which were the time of the current study, inadequate of the total heat for the ability of the parasite to complete its development in the invertebrate host (2), and geographic variation can theoretically be attributed to differences in exposure to parasite vectors and/or host susceptibility to parasites (62). The samples of doves and pigeons in this study were not migratory birds therefore may lead to decrease chance of infection (2, 63, 64). **Conclusions,** It is worthy to note that the current study is the first one which conducted to compare the infection rates of blood parasites between doves and domestic pigeons in Garmian administration. The result indicated that the two examined bird species were susceptible to be identified with *Haemoproteus columbae* protozoon parasite and the infection rate of them is relatively the same, therefore statistically no significant difference in the infection rate between them was found, these might be because of that the body resistance to this parasite in both bird species is

immunologically identical. In the current study there was no significant difference in infection rate between males and females and the number of infected birds in both sexes was almost equal. Microscopic examination of the Giemsa stained thin blood smears is the most reliable and cost effective method in diagnosing blood parasites. However, further studies are recommended applying molecular diagnostic techniques to easy identifying the species of haemosporidian parasites and to understand the ecological and evolutionary relationships between hosts, parasites and vectors in the region.

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