Research Article

AL- ANBAR JOURNAL OF VETERINARY SCIENCES

Vol. 14 Issue:1, (2021) ISSN: P-1999:6527 E-2707:0603

Identification of Blood Protozoa in See-See Partridges (*Ammoperdix Griseogularis)* in Garmian Area/Kurdistan Region-Iraq

Mohammed Abdulkareem Wahab[1], Shahnaz Abulkader Ali[2] and Nawzad Rasheed Abdulrahman[3]

[1] Veterinary Techniques Department/Kalar Technical Institute/Sulaimani Polytechnic University, Iraq.

[2]Microbiology Department/College of Medicine/Sulaimani University, Iraq.
[3] Dep. of Anatomy and Histopathology/College of Veterinary Medicine/Sulaimani University, Iraq.

*Corresponding Author: mohammed.wahhab@spu.edu.iq , 07701571521

Doi: https://doi.org/10.37940/AJVS.2021.14.1.5

Received: 12/2/2021 Revised:2/5/2021 Accepted:6/5/2021

Abstract

Haemosporidians are intracellular protozoan parasites found inside the blood cells and tissues of avian hosts; they are almost worldwide distributed and occur in a variety of avian species, including see-see partridge birds. Blood samples were collected from 63 see-see partridges during a period extended from 19th November 2018 to 15th March of 2019 in the Garmian area/Iraqi Kurdistan region to detect and investigate the infection rates of haemosporidian parasites by microscopic examination using Giemsa stained smears. The results of the study showed that out of the total samples numbers 63 only 43 were found infected with haemoprotozoan parasites composing the overall infection rate of 68,25%, from 32 birds which were infected with ***Plasmodium*** spp. at a rate 50,79%, and 8 birds were infected with ***Haemoproteus*** spp**.** at a rate of 12,69%., the results also revealed that the rates of infection with these protozoan parasites vary substantially among the sampled locations. The present study showed that two haemosporidian genera (***Haemoproteus*** spp. and ***Plasmodium*** spp.) were detected in see-see partridges in Kurdistan region-Iraq for the first time, and this type of bird is considered as a new host record for these haemoprotozoa in this area.

Keywards: Giemsa stain, *Haemoproteus* spp., *Plasmodium* spp., See-See Partridges

الكشف عن اوالي الدم في حجل الصفرد نوع (*Ammoperdix griseogularis*) في منطقة كرميان/إقليم كردستان العراق

الخلاصة

ان طفيليات الدم البوغية هي اوالي توجد داخل خلايا الدم وأنسجة الطيور حيث تنتشر في جميع أنحاء العالم تقريبًا وتتواجد في مجاميع متنوعة من أنواع الطيور بما في ذلك طيور حجل الصفرد .حيث تم جمع عينات دم من 63 طيور الحجل خلال الفترة الممتدة من 19 تشرين الثاني 2018 إلى 15 آذار 2019 في منطقة كرميان من إقليم كردستان العراق للكشف عن معدلات الخمج بطفيليات الدم البوغية والتحقق من وجودها عن طريق الفحص المجهري باستخدام صبغة الجيمزا. حيث أظهرت النتائج بأنه من إجمالي 63 عينة دم فقط 43 عينة ثبت خمجها بطفيليات الدم البوغية و بنسبة خمج كلي بلغ 68,25٪, حيث شملت 32 طائر مخمج بطفيلي الملاريا بنسبة 50,79% و 8 طيور مخمجة بطفيلي ***Haemoproteus*** spp. وبنسبة 12,69٪ . كما اوضحت نتائج الدراسة بان معدلات الخمج بهذه الطفيليات تختلف اختلافا كبيرا بين مناطق اخذ العينات ومن نتائج الدراسة ايضا تم اكتشاف جنسين من طفيليات الدم هما ***Plasmodium*** و ***Haemoproteus*** في الحجل في اقليم كردستان العراق لاول مرة ويعد هذا النوع من الطيور كمضيف جديد لهذه الطفيليات في هذه المنطقة.

Introduction

Haemosporidians are vector-borne eukaryotic protozoan parasites (Phylum Apicomplexa) that commonly found within the body cells of most bird species throughout the world. These protozoa have a global distribution and consist of four genera: *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, and *Fallisia*, they parasitize a variety of avian hosts including domestic chickens, ducks, geese, pigeons, doves, turkeys, game birds, owls, birds of prey, numerous passerines and parakeets, their severity of infections is different among various avian families, it is obvious that some avian hosts are more susceptible than others (1-3).

Avian haemosporidians usually cause losing weight, lowering productivity especially egg-laying in birds and lower development rates, in addition to the direct effect caused by anemia, and destruction of cell body and allergic response might occur (4). Few studies also have suggested that these types of haemosporidians infections can cause reduced speed and strength in flight, poor appetite, anemia, air sacculitis, and arthritis in hosts (5).

Iraq has rather a wide range of geological diversity, from the peaks of Kurdistan Mountains in the north to the deserts and semi-deserts in the west and the great Mesopotamian marshes in the south; these share in the enormous biological and avian diversity. See-see partridge (*Ammoperdix griseogularis*) is a game bird in the pheasant family Phasianidae of the order Galliformes (6), has a huge distribution range, from southeast Turkey through Syria and Iraq to Iran and Pakistan (7, 8), it is considered as one of the recorded birds in the checklist of Iraqi birds, where widespread breeding resident in hills and mountains in northern and eastern areas of this country (9). See-see partridge takes a great variety of seeds and some insect food; usually, it is seen in pairs or, at the most, in flocks of two to four birds. However, flocks have also been seen of more than fifty birds. When disturbed, the partridge prefers to walk rather than fly, but if needed it flies a short distance on rounded wings (10).

To date, *Haematozoa* of Iraqi birds are rather partially known based on many published studies on the different bird species usually comprising domestic chickens, pigeons, doves, ducks, geese, turkeys, passerines, chukars, partridges, quails, resident, wild birds, and migratory avifauna and some others, which were mostly infected with one or more species of *Haemoproteus*, *Plasmodium* and *Leucocytozoon* (5,11- 26). Even though much more researches had been carried out in various species of wild and domestic birds in Iraq by Mohammad *et al*. (16) and Shamsuddin and Mohammad (27) who were the first authors studied on see-see partridge birds in terms of *Haematozoan* parasites in this country the former found *Haemoproteus* and *Plasmodium* spp. with the rates of infection 30.9% and 2.4% respectively, and the latter recorded *Haemoproteus* spp. for instance with the rate 75%, after that, no work had been studied on this aspect in these birds**.**

So far due to a lack of knowledge concerning the distribution, prevalence, and identification of haemosporidian parasites, especially in see-see partridge in the Kurdistan region- Iraq, thus the objective of the current study was originally designed and conducted to isolate and detect various blood protozoan parasites in this type of bird within a given geographical or ecological unit in Garmian area/Iraqi Kurdistan region relating their prevalence of infection in these partridges.

Materials and methods

See-see partridge bird capturing

A total of 63 healthy adult see-see partridges of different ages, including both sexes were

captured randomly from the period between November 19th 2018 to March15th of 2019 by using a fine mesh net in various locations of Garmian area, its capital is Kalar district which is located at 150 Km. southeast of Sulaimani city center, in the southeast Kurdistan region of Iraq. It is in between the latitudes (34˚15 33 = - 35˚ 11– 05 =) above the equator and the longitudes (44˚ 29–41 = - 45˚ 54– 20 =) of the eastern hemisphere. The Garmian involves the districts: Kalar, Kifri, and Khanaqin, and its total area are 6731.73 square kilometers (28) (Fig. 1).

After the partridges being captured, they were transferred to the parasitological laboratory of Kalar Technical Institute /Sulaimani Polytechnic University and kept them alive in separate cages, then tagged with a leg ring to avoid duplicate sampling.

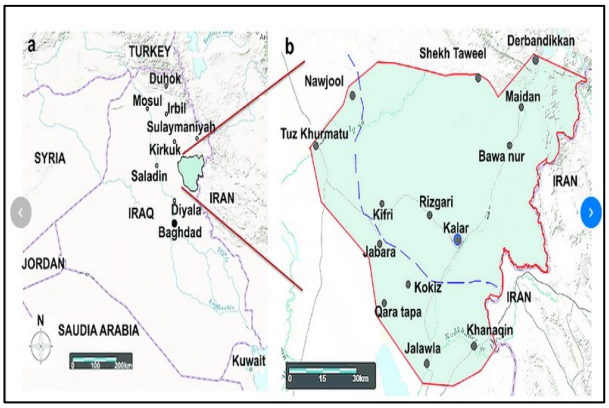

Figure 1. Garmian Area map, Geographical map of the study area around the Garmian area (28).

Blood samples collection and preparation of Giemsa-staining blood smears

After the wing vein of each sampled bird was disinfected by 70% ethyl alcohol, a small amount of blood was oozed by puncturing the vein with a sterilized disposable lancet (29), then the blood smears were prepared as suggested by Sriraman (30) as follows: A small drop of blood was placed on the end of a clean glass slide, then the blood drop was spread by the end of another glass slide which was placed in front of the drop at an angle of approximately 30 degrees and pushed back until it just touched the drop, immediately after that, the spreader slide was pulled forward smoothly and quickly at the same angle, the blood smears were air-dried by waving the slide in the air, then all slides were labeled with specific numbers and kept in box slides until the time of staining.

Staining of blood smears for the detection of haemosporidian parasites

The blood smears were fixed in absolute methanol for 3 minutes, after they were air dried, they immersed in working Giemsa staining solution 1:10 at pH 7-7.2 for 30 minutes, directly after staining, then were washed in distilled water, and air dried finally, examined microscopically using an Olympus BX43 light microscope; at least 100 fields were examined under 400X and oil immersion (1000×) magnification (30), morphological identification of detected parasites and red blood cells was performed following the taxonomic keys of (24, 31, 32).

Results and discussions

An examination of the blood smears indicated the existence of different intracellular stages of haematozoan parasites consistent with Plasmodium spp. and Haemoproteus spp. (Fig. 2: A, B, C, and 4). Out of 63 microscopic examination of the stained thin blood smears from different ages and sexes of healthy adult see-see partridges 68.58% was an overall infection rate in which Plasmodium parasites were the more frequent blood parasite detected in the present study, thus it was clear that 32 birds were found infected with Plasmodium spp. with an overall prevalence rate of 50.79% (Table 1), these protozoa were characterized by intra-erythrocytic halter-like gametocytes together with signet ring-shaped meronts (Fig. 2 A) during the microscopic examination of the Giemsa

stained blood smears of infected bird with Plasmodium parasites, in which morphological alterations and nuclear displacement were noticed in some erythrocytes because of the presence of these gametocytes and meronts (Fig. 2: A, B and C).

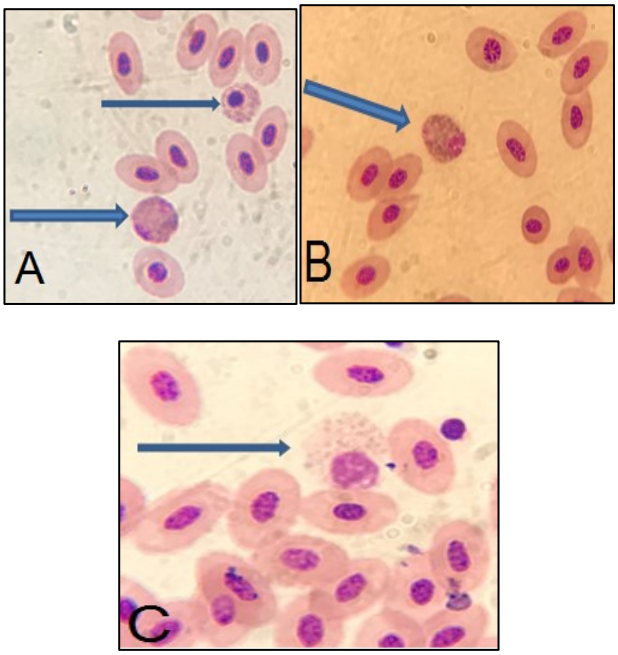

Figure 2. (A): Intra-erythrocytic forms of Plasmodium spp with mature gametocyte (arrows), in see-see partridge (X 1000), (B): Intra-erythrocytic meronts of Plasmodium spp. (arrow) in see-see partridge (X 1000), (C): Mature schizont of plasmodium spp. (arrow) in see-see partridge (X 1000).

The findings as well as showed that 8 examined birds were found infected with Haemoproteus spp. parasites, with an overall infection rate of 12.69% (Table 1). The microscopic examination of blood smears of the examined birds infected with Haemoproteus parasites revealed the presence of intra-erythrocytic gametocytes occupying about one-half of the erythrocyte cytoplasm with little or no displacement of the host cell nuclei; they surround the nuclei either partially or completely, also, brown pigment granules from digestion of host hemoglobin (hemozoin pigment granules) are evident within the infected erythrocytes (Fig. 3). It is worthy to mention that this study recorded a new bird host which is see-see partridge for the haematozoan parasites such as haemoproteus spp. and plasmodium spp.

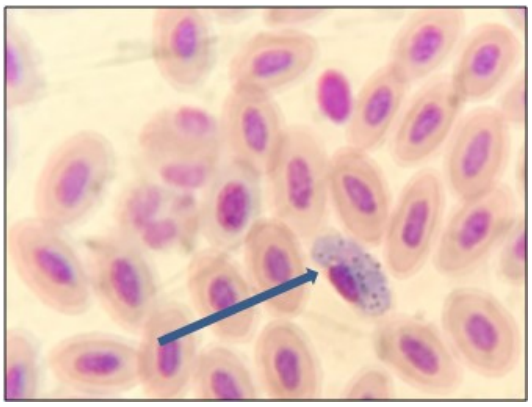

Figure 3. Intra-erythrocytic Haemoproteus spp. gametocyte (arrow), partially surrounds the nucleus of the host cell in see-see partridge (X 1000).

Three positive cases were revealed mixed infected from overall examined blood smear samples with Plasmodium and Haemoproteus parasites with an infection rate of 4.76%. As shown in Table 1, the rates of single infection showed that the plasmodium parasite was higher in rate (50.79%) than that of Haemoproteus (12.96%), it is also clear that the mixed infection rate with plasmodium and Haemoproteus spp. 4.76% was lower in the rate of infection. Haemosporidians are one of the most highlighted and well-studied protozoan parasites due to their actual relationship as causative agents of human malaria (30).The current study was based on Giemsa stained blood smears for the detection of haemosporidian parasites in see-see partridge in Garmian area/ Kurdistan region-Iraq. Up to date, research works in the Kurdistan region generally have been looking for the infection of blood protozoa in chickens, passerines, chukars, pigeons, doves, turkeys, and other resident birds, however, no efforts have been carried out to identify the existence of haematozoan parasites in

see-see partridges, and this was the aim of the current study.

Table 1. Plasmodium and Haemoproteus parasites species and their infection rates in see-see partridge bird

No. of examined birds	Detected parasite genera	Positive cases (+ve)	Infection rates (%)
63	*Plasmodium* parasite	32	50.79%
	Haemoproteus parasite	8	12.69%
	Mixed infection (*Plasmodium and Haemoproteus parasites)*	3	4.76%
Total		43	68.25%

As much as we know, this study is the first attempt to detect and investigate the infection rates of haemosporidian in this type of bird in the Iraqi Kurdistan region.

The interpretation of this result is somehow similar to those shown by (33, 34) in which; some avian species appeared to be more susceptible than others. For instance, ducks, geese, and swans are commonly infected with species of Haemoproteus, Leucocytozoon, and Plasmodium, and more than 75% of waterfowl species were hosts for one or more of these parasites, it is obvious that pigeon and dove species have similarly high rates of infection, but genera of other families, such as migratory shorebirds, are less frequently parasitized.

From the results, out of the 63 see-see partridges involved in this study, only 43 of them were found infected with haemosporidian parasites. As an Iraqi area, Garmian is a subtropical region accordingly the infection rate (68.25%) reported in this study appears to be variable in comparison with infection rates reported in birds in the nearby tropical and subtropical regions (35, 36) and in subtropical regions around the world (37).

So far, (16, 27, 38) who were the first authors studied

on see-see partridge birds in terms of Haematozoan parasites in this country. Bennet et al. (38) found that 44 out of 185 species of Phasianidae examined internationally were infected with Haemoproteus spp. reporting of Haemoproteus danilewskyi constitutes the record for Iraq while Haemoproteus santosdiasi was reported by Shamsuddin and Mohammad (27) from the same host, the see-see partridge. Mohammad et al. (16) recorded Haemoproteus and Plasmodium spp. in Iraq with the rates of infection 30.9% and 2.4% respectively, their haematozoan infection rates actually varied with those of the current work (Table 1), often these variations might be influenced by many real factors related to the variation in the incidence or prevalence like health status of bird, sex, age, feeding and feeding habitats, season, climatic variables such as temperature and the presence of transmitters (39).

As well as the high infection rate (68.25%) of haemosporidian parasites in the present study might be stemmed from different related reasons as mentioned above. The first reason is the time of sampling. The blood samples of see-see partridge were collected during the period extended from November 19thof 2018 to March 15th of 2019. This period was almost wet and thus the proliferation of insect vectors could be largely activated, so this finding contrasts with many observational reports that pointed out a low prevalence of haemosporidian parasites in birds sparsely exposed to vectors (30, 40). In fact

increased vector exposure leading to a high risk of infection by haemosporidian parasites is associated with wet seasons (40, 41), tropical zones (42), and forest habitats (43).

The most predominant parasite genus encountered in the current study was Plasmodium (50.79%). The rate of these parasites (Plasmodium spp.) was approximately 4 fold higher than the rate of Haemoproteus ones, such differences in the relative proportion of the two genera of haemosporidian parasites have also been observed by other authors Mohammad, et al. (16) and Bennett et al. (33) who suggested important differences in biological transmission such as host and vector behavior and density, for example, black flies typically require flowing water for larval development (36).

Generally, the pathogenesis of parasitic diseases depends upon many factors such as the age of the host species and its health status, site of infection, and numbers of parasites (44), however, no pathological signs were observed in the partridges examined in this work.

Conclusions

It was concluded from the present study that see-see partridges might be infected with Plasmodium and Haemoproteus blood parasites in Garmian area/ Kurdistan Region-Iraq. The results of the present study explored for the first time the existence of the Plasmodium spp. and Haemoproteus spp. in the partridges in this area. Really the various geographical areas with different climatic changes are the most powerful impact on the rates of infection with these haematozoa. In this work, the rate with Haemoproteus spp. parasite was lower in comparison with plasmodium one. As it was recorded, the birds were really susceptible to single and/or mixed haematozoan infection with various rates, and this might become a source of infection to domestic and wild birds in our region through the distribution of vectors and lead to an economic loss of poultry in our country.

References

1. Dey AR, Begum NM, Anisuzzaman AH, Mondal MM. Haemoprotozoan Infection in Ducks Prevalence and Pathology. Bengladish Journal of Veterinary Medicine. 2008; 6: 53-58.

2. Swayne DE. Diseases of Poultry. 13th Edition. Blackwell Publishing Ltd; 2013.

3. Vaclav B, Vlastimil B, Miroslav C, Martin H, Ivan L. Blood parasites (Haemoproteus and microfilariae) in birds from the Caribbean slope of Costa Rica. W. Stefan´ski Institute of Parasitology, PAS Acta Parasitologica. 2009; 54 (3): 197–204.

4. Archawaranon M, Subinprasert S. Bird-parasite relations: A hill mynah case study. J. Entomol. 2005; 2 (1): 112-116.

5. Remple JD. Intracellular Hematozoa of raptors: a review and update. J. Avian. Med. Surg. 2004; 18: 75–88.

6. Mohammad MK. Haemoproteids of the avian family Rallidae in Iraq with description of a new species. Bull. Iraq nat. Hist. Mus. 2001; 9 (3): 51-56.

7. Grimmett R, Inskipp C, Inskipp T. Birds of the Indian Sub-continent. Helm Publications, London: UK; 1998.

8. Roberts TJ. The birds of Pakistan. Oxford University Press, Karachi, Pakistan; 1992.

9. Mohammad KM, Al- Moussawi AA. Haematozoa of Resident Urban Birds of Iraq. Adv. Biore. 2013; 4 (3): 54-57.

10. Khaliq I, Muhammad B, Maria R, Aleem AK. Genetic diversity in see-see partridge

(Ammoperdix griseogularis, Galliformes) populations from sub-Himalayan Mountain ranges of Pakistan, Belg. J. Zool. 2010;140 (2): 229-234.

11. Abdulla SH, Mohammed AA, Saeid NM. Study of Ecto and Haemo parasites in domestic pigeons (Columbia livia) in Sulaimani Province, Kurdistan region/Iraq. Journal of Zankoy Sulaimani. 2018; 20 (1), (part A): 37-44.

12. Ali KN, Nawzad RA, Shahnaz AA. Prevalence of Haemosporidian parasites of Chukar Patrtridge Birds in Sulaimani Province /Kurdistan Region of Iraq. Al-Anbar lournal of Veterinary Sciences. 2019; 12 (2): 81-88.

13. Ali SA, Wahhab MA, Abdulrahman NR. Detection of Haemoproteus columbae in Feral Pigeons in Sulaimani Province, Iraqi Kurdistan Region. Al-Anbar J. Vet. Sci. 2017; 10 (1): 29-33.

14. Hasson RH. Haemosporidians parasites of Gallus domesticus , poultry in Iraq. International Journal of Advanced Research. 2015; 3 (8), 1046 – 1054.

15. Ibrahim RM, Al-Rubaie HM. Prevalence of some Haemosporidians in domesticated chickens in Baghdad city. Plant Archives Vi. 20 Supplement. 2020; 1: 3444-3448.

16. Mohammad KM, Jasim MK, Al-Moussawi AA. Haematozoa of the Avian Family Phasianidae in Iraq. Bull. Iraq nat. Hist. Mus. 2001; 9 (3): 57-61.

17. Mohammad MK. Blood parasites of some Iraqi wild birds. Iraqi J. Sci. 1990; 31(Supplement):31-39.

18. Mohammad MK. Haemoproteus burhinus A new species from the stone curlew Burhinus oedicnemus saharae (Reichenow) in Iraq. Bull. Iraq nat. Hist. Mus. 1996; 8 (4): 103-111.

19. Mohammad MK. Blood parasites of the babblers of Iraq. Bull. Iraq nat. Hist. Mus. 2002; 9 (4): 33-40.

20. Mohammad MK. Haematozoa of the grey hypocolius Hypocolius ampelinus Bonaparte (Aves: Hypocoliidae) in Kerbala Province, Middle of Iraq. Bull. Iraq nat. Hist. Mus. 2003; 10 (1):49-57.

21. Mohammad MK, Al-Naeimi TM. Blood parasites of two bee-eaters in Iraq. Bull. Iraq nat. Hist. Mus. 2000; 9 (2): 71-77.

22. Mohammad, MK. The haemoproteids of the avian family Scolopacidae in Iraq with description of a new species. Bull. Iraq nat. Hist. Mus. 2004; 10 (2): 57-63.

23. Mohammed NH. Study on the blood protozoa in geese. Iraqi Journal of Veterinary sciences. 2020; 34 (1): 23-27.

24. Soulsby EJL. Helmonths, Arthropods and Protozoa of Domesticated Animals. 7th Ed. Bailiere Tindalland Cassel. LTD. London: 1982; 763-765.

25. Wahhab MA, Ali SA, Abdulrahman NR. A Comparative Study of Blood Parasites Naturally Occurring in Doves and Domestic Pigeons in Garmian Area-Iraqi Kurdistan Region. Al-Anbar J. Vet. Sci. 2017; 10 (1): 20-28.

26. Zangana MF. Study on the parasites of domestic pigeon Columba livia domestica in Nineva and some areas of Erbil and Duhouk Provinces. M. Sc. Thesis, College of Science, University of Mosul, Iraq; 1982.

27. Shamsuddin M, Mohammad MK. Haematozoa of some Iraqi birds with description of two new species Haemoproteus pteroclis and Leucocytozoon nycticoraxi (Protozoa: Haemosporina). Bull. Nat. Hist. Res. Centre. 1981; 7 (4): 111- 154.

28. Al-Bajalan MM, Al-Jaf SMA, Niranji SS, Abdulkareem DR, Al-Kayali KK, Kato H. An outbreak of Leishmania major from an endemic to a non-endemic region posed a public health threat in Iraq from 2014-2017: Epidemiological, molecular and phylogenetic studies. PLoS Negl Trop Dis. 2018; 12 (3): 1-11.

29. Eljadar M, Saad W, Elfadel G. A study on the prevalence of endoparasites of domestic Pigeons (Columba livia domestica) inhabiting in the Green Mountain Region of Libya. J. American Sci. 2012; 8 (12): 191-193.

30. Sriraman PK. Veterinary laboratory diagnosis. Jaypee Brothers Medical Publishers, New Delhi; 2009.

31. Valkiunas G. Avian Malaria Parasites and Other Haemosporidia, CRC Press, Boca Raton, Florida, USA; 2005.

32. Valkiūnas G, Tatjana AI. Keys to the avian malaria parasites. Valkiūnas and Iezhova Malar Journal. 2018; 17:212.

33. Atkinson CT, VanRiper CIII. Pathogenicity and epizootiology of avian haematozoa: Plasmodium, Leucocytozoon, and Haemoproteus, in Loye, J.E., and Zuk, M., eds., Bird-parasite interactions, Ecology, evolution, and behavior: New York, Oxford University Press. 1991; 19–48.

34. Bennett GF, Montgomerie R, Seutin G. Scarcity of haematozoa in birds breeding on the arctic tundra of North America. The Condor. 1992; 94: 289–292.

35. Ishtiaq F, Gering E, Rappole JH, Rahmani AR, Jhale YV, Dove CJ, Milensky C, Olson SL, Peirce MA, Flischer RC. Prevalence and Diversity of Avian Hematozoan Parasites in Asia: A Regional Survey. Journal of Wildlife Diseases. 2007; 43: 382-398.

36. Martinsen ES, Blumberg BJ, Eisen RJ, Schall JJ. Avian hemosporidian parasites from Northern California oak Woodland and Chaparral Habitats. Journal of wildlife diseases. 2008; 44: 260-268.

37. Forrester DJ, Telford SR, Foster J, Bennett GF. Blood parasites of raptors in Florida. Journal of Raptor Research. 1994; 28:226–231.

38. Bennett GF, Whiteway M, Woodworth-Lynas CB. Host-parasite catalogue of the avian haematozoa: Memorial University of Newfoundland Occasional Papers in Biology. 1982; 5: 243.

39. Senlik B, Gulegen E, Akyol V. Prevalence and intensity of Haemoproteus columbae in domestic pigeons. Indian Vet. J. 2005; 82: 998-9.

40. Young BE, Garvin MC, McDonald DV. Blood Parasites in Birds From Monteverde, Costarica.Journal of Wildlife Diseases. 1993; 29: 555-560.

41. Deviche P, Greine EC, Manteca X. Seasonal and age-related changes in blood parasite prevalence in Dark-eyed Juncos (Junco hyemalis, Aves, Passeriformes). J. Exp. Zool. 2001; 289:456-66.

42. Durrant KL, Beadell JS, Ishtiaq F, Graves GR, Olson SL, Gering E, Peirce MA, Milensky CM, Schmidt BK, Gebhard C, Fleischer RC. Avian haematozoa in South America: a comparison of temperate and tropical zones. Ornithological Monographs. 2006; 60: 98-111.

43. Garamszegi LZ. Climate change increases the risk of malaria in birds. Glob Change Biol. 2011; 17:1751–9.

44. Belding DL. Textbook of parasitology, 3rd ed. Meredith Publishing Company, New York: 1965; 1374.