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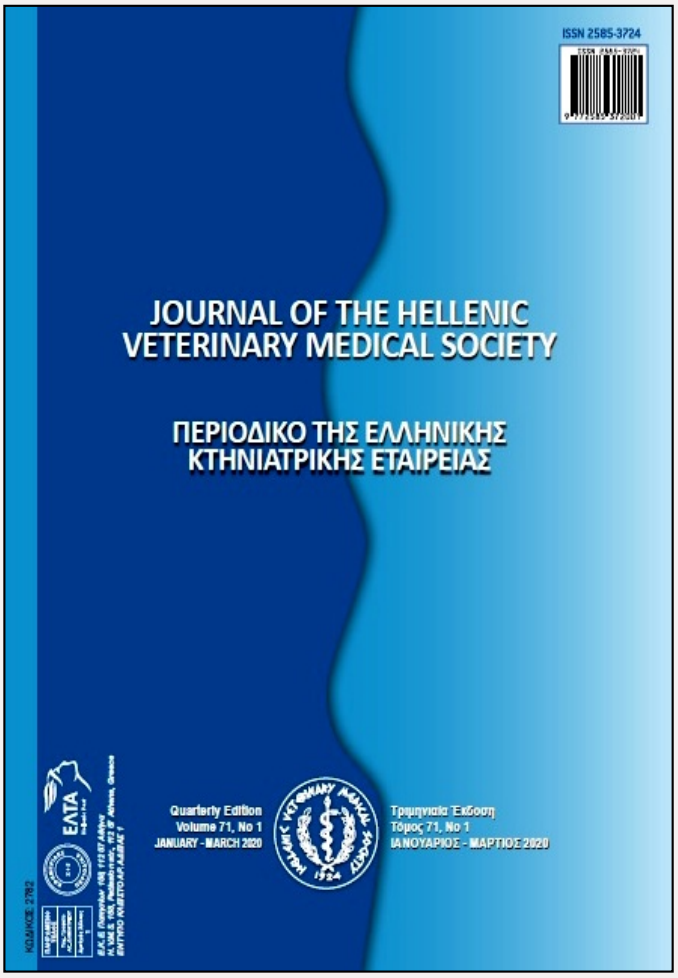


# Prevalence and new histopathological aspects of Haemoproteus spp. in pigeons from Iran

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# Prevalence and new histopathological aspects of *Haemoproteus spp.* in pigeons from Iran

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**ABSTRACT.** *Haemoproteus spp.* is pathogenic protozoan that effecting blood circulatory system of birds. The present study was undertaken to evaluate the presence of *Haemoproteus spp.* in pigeons from Iran and associated histopathological changes. A total of 108 blood samples were taken from pigeons to investigate *Haemoproteus spp.* presence by blood smear and semi-nested PCR targeting the cytochrome b gene methods. Also, to evaluate histopathological changes 12 infected pigeons to *Haemoproteus* were sacrificed and studied. 34.2% of pigeons infected with *Haemoproteus* showed macro and microgametocytes in their erythrocytes while based on the molecular method 63.8% were infected. Focal lymphocytic aggregates, pigmentation and cell swelling were the main histopathological lesions in infected livers. Multifocal non- suppurative interstitial nephritis, pigmentation and splenic lymphoid hyperplasia were also seen in the infected pigeons. Mild lymphocytic myocarditis in the heart of one pigeon was the other finding. No histopathological changes were seen in brain, intestine, and pancreas. Schizonts with variable shapes and sizes were detected in infected livers, lungs, kidneys, and spleens but megaloschizonts were not found. This study also reports the molecular prevalence of *Haemoproteus spp.* in Iran

***Keywords:*** *Haemoproteus spp*., Pigeon, Semi- nested PCR, Histopathology, Iran

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## INTRODUCTION

Species of the apicomplexans *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* comprise a diverse group of vector transmitted parasites that infect red blood cell (in the case of *Leucocytozoon spp*., also white blood cells) and other organs within their vertebrate hosts (Atkinson & Van Riper, 1991; Valkiūnas 1993). These parasites have served as model organisms for studies on many aspects of parasite-host interactions, including parasite-host evolution (Perkins & Schall, 2002; Ricklefs & Fallon, 2002), host life-history trade-offs (Richner et al., 1995), and sexual selection (Hamilton & Zuk, 1982).

Haemosporidian protozoa of the genus *Plasmodium* (Marchiafava et celli, 1885) and *Haemoproteus* (Kruse, 1890) have a broad geographic distribution and a diversity of vectors and have been described throughout the world as parasitizing several hosts, including birds, mammals, and reptiles (Garnham 1966; Valkiūnas 2005). In birds, these parasites, has been described as a potential cause of extinction and population decline (Van Riper III et al. 1986, Atkinson et al. 1995, 2000, Massey et al. 1996), reducing the fitness of their hosts (Lefèvre et al., 2008) and may sometimes lead to death (Donovan et al. 2008, Cannell et al. 2013). Severe infections by haemosporidian can lead to death and involve different physiopathological phenomena such as anemia, thrombocytopenia, and inflammation (Macchi et al., 2013; Cannell et al., 2013).

*Haemoproteus spp.* is characterized by schizogony (i.e., merogony) within visceral endothelial cells, typically of the lung, liver, or spleen, and gametocyte development in circulating erythrocytes (Bermudez 2003; Campbell 1995). The organisms are transmitted by biting flies, characteristically louse flies (Hippoboscidae) and biting midges (Ceratopogonidae). Sexual stages of *Haemoproteus spp.* occur in the intermediate host (i.e., insects) with asexual stages in the bird. *Haemoproteus spp.* infection is usually subclinical but can cause mild clinical signs (Earle et al., 1993; Macwhirter 1994; Merino et al., 2000). When clinical disease occurs, it is typically associated with anemia because of erythrocytic parasitism, (Cardona et al., 2002) frequently in immunocompromised hosts. The true extent of pathology and mortality caused by *Haemoproteus spp.* parasites remain unclear because the severe hemoproteosis and death of infected birds occur mainly during the tissue stage of parasite development, before the appearance of parasitemia. Recent PCR- based findings indicate that species of *Haemoproteus* are responsible for some instances of mortality in birds. Due to the application of molecular diagnostic methods, the traditional opinion about the harmlessness and insignificant veterinary importance of avian hemoproteids is an ongoing partial reconsideration. *Haemoproteus spp.* parasites are worth more attention in veterinary medicine and in bird conservation projects (Valkiūnas 2015). Natural infection with *Haemoproteus spp.* has been reported in pigeons from Iran. For example, Tavassoli et al., (2017) examined 93 blood samples of pigeons for *Haemoproteus spp.* infection. In their study, 13 (13.97%) samples were positive in stained blood smears while 27 (24.73%) were positive by PCR. In Nourani et al., investigation (2018), 37.5% of passerine birds from the East of Iran have been detected as harboring Haemoproteus spp.

The distribution and nature of histopathological lesions and the types of schizonts present in the tissues in different species of *Haemoproteus* vary in reports published to date.

In this study, in addition to the evaluation of *Haemoproteus spp.* prevalence in Iranian pigeons, the aim was to investigate histopathological changes of different tissues. Possible existence of megaloschizonts in different tissues infected with *Haemoproteus spp.* was investigated. Some new findings in histopathological changes in pigeons infected with *Haemoproteus spp.* are discussed.

## MATERIALS AND METHODS

The study was conducted in Khuzestan, a south-western province of Iran from November 2015 and November 2016. Khuzestan province has an area of about 64 236 km$^2$ (Statistical book of Khuzestan province, 2006). The province has hot and wet summers, a mild spring and cold winters.

### Preparation of blood smears and morphological analysis

For the observation of blood hemoparasites, samples were collected from 108 pigeons between November 2015 and November 2016. The volume of blood collected was almost 50 µL, not exceeding 1% of the live weight of the animal, as recommended by SISBIO, Campbell (1995) and Clark et al., (2009). The blood was collected by puncture of the brachial vein and was used for the preparation of blood smears and/or for PCR testing. Thin blood smears were prepared, fixed with absolute methanol (1 min), stained with 10% Giemsa solution (30 min) and examined under oil immersion lens. More than 60 microscopic

fields of blood films at a magnification of ×1000 were examined. When no parasite was observed, another smear was examined to confirm the result. Length and width of infected and non- infected RBCs were recorded. Also, length, width, and number of granules in macro- and microgametocytes were evaluated. The infection intensity was estimated by scoring the infections as weak (<10 parasites/1000 RBCs), moderate (10-100 parasites/1000 RBCs), and severe (>100 parasites/1000RBCs) infections.

**DNA extraction and molecular analysis**

DNA was extracted using a genomic DNA purification kit (Cinna Gen, Iran). Partial amplification of a 390-base-pair (bp) fragment of the cytochrome b (cyt b) gene of the parasites was accomplished by PCR using the nonspecific primers PALU-F (59-GGGTCAAATGAGTTTCTGG-39) and PALU-R (59-DGGAACAATATGTARAGGAGT-39) as described in Martınez et al., (2009). This set of primers is unable to distinguish between *Plasmodium* and *Haemoproteus* genera, so to confirm mixed infections formed by both *Haemoproteus* subgenera, the following forward primer PALU-F1 (59-TAGTTAGCGACCCAACAC-39) was designed and used with the reverse primer PALU-R, to amplify specifically a DNA fragment of 301 bp from the *Haemoproteus* subgenera. PCR reactions included a negative control, consisting of the reaction mix and 2 µl of DNase/RNase-free water and a positive control that consisted of a DNA sample from the blood of a pigeon with positive blood smear. All PCR reactions were performed in a 20 µl mixture consisting of 10 µl Taq master mix, 1 µM primers and 5 µl DNA template. PCR cycling included an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 45 s. This was followed by a final extension step at 72 °C for 10 min. At least 20% of the samples were randomly selected, and the amplification reaction was repeated to ensure the reproducibility of the technique. PCR products were electrophoresed in 1.5% agarose in Tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide to visualize the amplified DNA fragments under ultraviolet light. The samples were assigned to: (1) Parahaemoproteus subgenus when the results were positive for the first set of primers and negative for the second set, (2) *Haemoproteus* subgenus when the results were the opposite (negative and positive, respectively), and (3) unknown when both sets of primers yielded a positive result. Amplified fragments corresponding to the expected size taken from five pigeons were purified using a PCR purification kit (Vivantis, Revongen Corporation Center, 47600 Subang Jaya, Selangor Darul Ehsan, Malaysia). The PCR fragments were sequenced using specific primers and the Big Dye Terminator V.3.1 Cycle Sequencing kit in an ABI 3130 Genetic Analyzer (Applied Biosystems, 850 Lincoln Centre Drive Foster City, CA 94404, USA). Multiple sequence alignment analysis between sequences taken from samples and those from submitted to the GenBank (http://ncbi.nlm.nih.gov) was performed using nBLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

**Histopathological analysis**

In this study, we tried to investigate histopathological changes in pigeons that were apparently healthy, and they did not show any clinical symptoms of bacterial or viral infections. Also, any findings that were suspicious of other infections were excluded from the study. Finally, twelve pigeons with positive blood smears the infections of which confirmed with the molecular method were selected for histopathological studies. Pigeons were euthanized in a glass desiccator jar for open-drop anesthesia with chloroform following standard animal ethics guidelines of Iran. All experiments were performed according to the requirements of the animal welfare committee of Shahid Chamran University of Ahvaz following the Iranian Veterinary Medical Association guidelines. For histopathological analysis, tissue samples were taken from liver, heart, lung, kidney, spleen, brain, intestine and pancreas. The samples were fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, embedded in paraffin wax, sectioned at 5 µm, stained with hematoxylin and eosin and Congo red, and studied with a light microscope.

**Statistical analysis**

Student T. test was used to compare changes of length and width of infected and non- infected erythrocytes and their nucleus. *P*-values of $< 0.05$ were considered statistically significant.

## RESULTS

**Blood smear examination**

A total of 108 thin blood smears were examined and 37 (34.2%) were infected with *Haemoproteus spp.* macro and microgametocytes. The intensity of the parasitemia was 1-254 infected cells in 60 microscopic fields. Among the infected pigeons 14, 19 and four showed weak, moderate and severe infections, respectively (Fig 1).

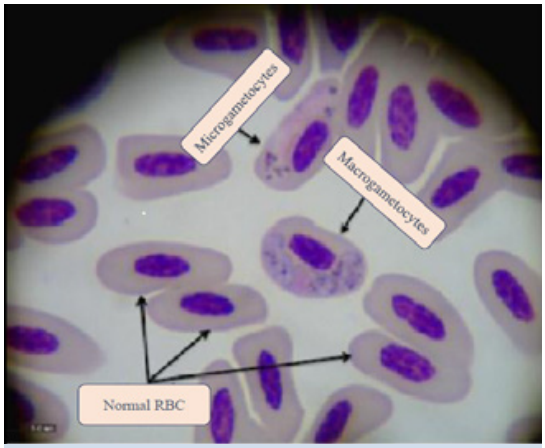


**Fig 1.** Normal RBCs, macrogametocytes and microgametocytes of ***Haemoproteus spp.*** in the blood smears of infected pigeons.

Length and width of infected and non- infected erythrocytes and their nucleus are presented in Table 1. There were not significant differences in the length of infected erythrocytes and their nucleus but there were significant differences in the width of erythrocytes infected with macro (P=0) and microgametocytes (P=0.03) in comparison with non- infected RBCs. Furthermore, nucleus width of erythrocytes infected with macro (P=0.001) and microgametocytes (P=0.004) were significantly decreased. Length, width and number of granules were 14.26 µm, 3.18 µm, 23.86 µm and 11.91 µm, 3.13 µm and 8.43 µm in macro and microgametocytes, respectively. Table 2 showed the mean of length, width and number of granules found in *Haemoproteus spp*. macro and microgametocytes.

**Table 1.** Length and width of infected and non- infected RBCs by *Haemoproteus spp.* in pigeons. Results are expressed as mean ± SD.

| | **Non infected RBC** | | **Infected RBC with macrogametocytes** | | **Infected RBC with microgametocytes** | |
|---|---|---|---|---|---|---|
| | Cell | nucleous | Cell | nucleous | Cell | nucleous |
| Length (µm) | 13.4 ± 0.7 | 6.7±0.5 | 14.2±1.3 | 6.6±0.6 | 13.7±0.9 | 6.7±0.4 |
| Width (µm) | 6.7±0.6 | 3.06±0.3 | 7.6±0.5 | 2.6±0.2 | 7.33±0.5 | 2.6±0.2 |

**Table 2.** Length, width and number of granules of *Haemoproteus spp.* in pigeons. Results are expressed as mean ± SD.

| | **Length** | **width** | **No. granules** |
|---|---|---|---|
| **Macrogametocytes (µm)** | 14.2±1.7 | 3.1±0.4 | 23.8±9 |
| **Microgametocytes (µm)** | 11.9±1.4 | 3.1±0.3 | 8.4±2.6 |

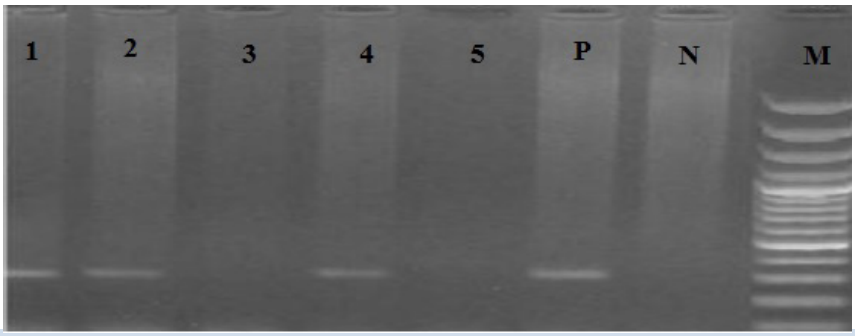


**Fig 2.** Amplification of *Haemoproteus spp.* DNA. Lane M is a 100-bp ladder. Lane P is a positive control DNA lane. Lane N is a negative control DNA lane. Lanes 1, 2, and 4 represent positive field samples. Lanes 3 and 5 represent negative field samples.

**Molecular analysis**

In 69 samples of these 108 examined samples, the PCR was positive and a band of approximately 300bp was seen on the agarose gel which considered as infection with *Haemoproteus spp*. (63.8%) (Fig. 2). All sequenced samples were found by BLAST analysis to be closest to the *Haemoproteus spp.* gene in GenBank with a similarity of ≥ 98%.

**Pathological findings**

The gross pathological changes of the liver were characterized by darkening and enlargement of the organ. Histopathologically, focal hepatic lymphocytic aggregations with variable sizes were seen in infected birds (Fig 3). Also, mild infiltration of heterophils was seen in the aggregates in three samples. Based on the number of aggregations, liver involvement was

categorized in weak (1-3), moderate (4-7) and severe (>7). One, five and six livers had weak, moderate and severe lymphocytic aggregations. Furthermore, in the most severe cases, accumulation of lymphocytes was also seen in many sinusoids. Hepatic pigmentation was detected in all infected pigeons. Yellow to dark brown refractile intracytoplasmic particles were detected in RBCs, endothelial cells, Kupffer cells, leukocytes and hepatocytes (Fig 4). Cell swelling, macrovesicular lipidosis, multifocal heterophilic hepatitis (Fig 5) and telangiectasis were other findings. Hepatc cirrhosis was diagnosed in one of the infected birds. Fibrosis, biliary ducts hyperplasia, nodular regeneration of hepatocytes, pigmentation, lipidosis, diffuse cell swelling and infiltration of heterophils, macrophages and lymphocytes were seen in this specimen. Different microscopic lesions of livers and their frequencies are presented in Table 3.

**Table 3.** Frequency of histopathological lesions found in liver of pigeons infected by *Haemoproteus spp.*

| Histopathological lesions | Frequency (%) |
|---|---|
| Focal lymphocytic aggregates | 12 (33.3) |
| Pigmentation | 12 (33.3) |
| Cell swelling | 8 (22.2) |
| Lipidosis | 1 (2.8) |
| Multifocal heterophilic hepatitis | 1 (2.8) |
| Telangiectasis | 1 (2.8) |
| Cirrhosis | 1 (2.8) |
| Total | 36 (100) |

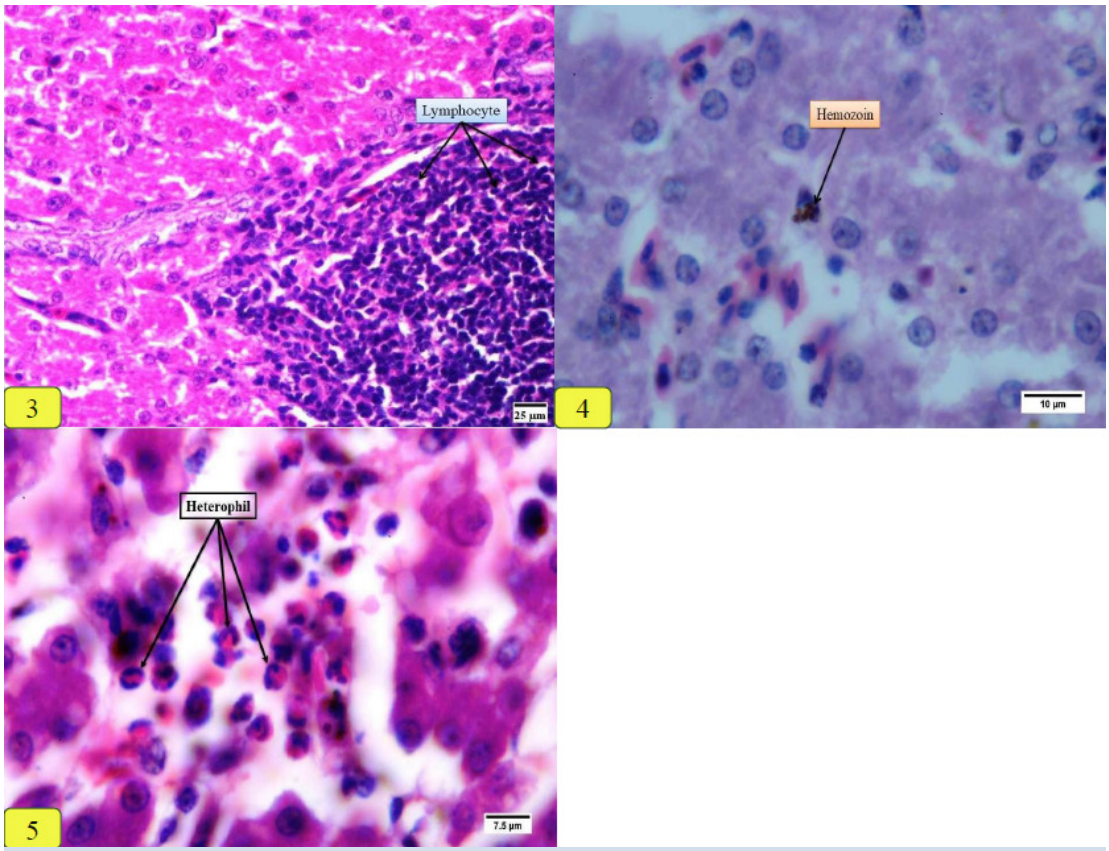


**Fig 3-5.** Cross sections of pigeon's liver infected by *Haemoproteus spp.* (H&E). (3) Tissue section indicating focal lymphocytic aggregation. (4) Tissue section indicating hemozoin pigment in a Kupffer cell within a sinusoid. (5) Tissue section showing focal heterophilic hepatitis.

There were no obvious gross changes in kidneys, but histopathological study showed multifocal non-suppurative interstitial nephritis (Fig 6).

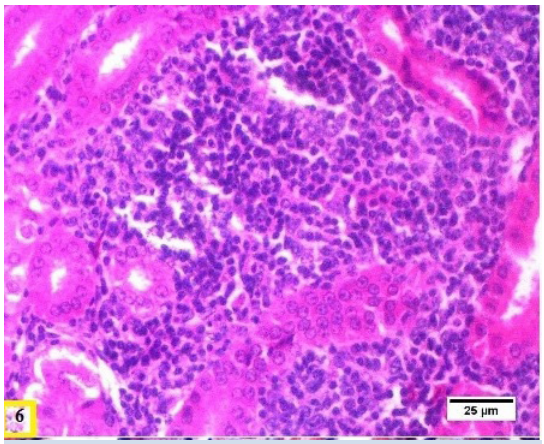


**Fig 6.** Tissue sections of pigeon's kidney infected by *Haemoproteus spp.*. (6) Tissue section showing non- suppurative interstitial nephritis with infiltration of mononuclear leukocytes (mainly lymphocytes) between tubules (H&E).

Table 4 represents kidneys histopathological changes with their frequencies. Pneumoconiosis was diagnosed in the lungs. Table 4 represents pulmonary histopathological changes and their frequencies. Various degrees of splenomegaly and darkening were noted in spleens in gross study. Severe (6 samples) and moderate (6 samples) hyperplasia of splenic white pulp accompanied with pigmentation were detected in 12 infected animals (Fig 7,8). Also, white pulps contained lymphoid follicles that were dilated or transformed into hyaline blocks. In heart of one infected pigeon mild lymphocytic myocarditis was detected. Brain, intestine and pancreas had no gross or histopathological changes.

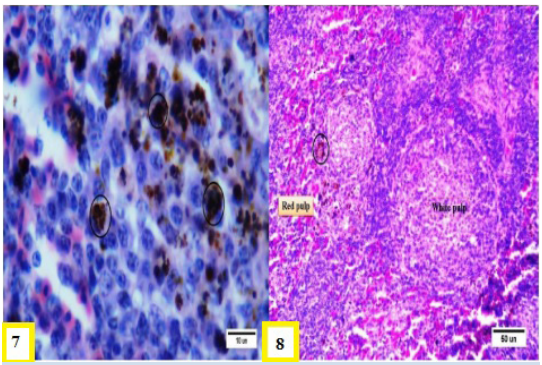


**Fig 7 and 8.** Cross sections of pigeon's spleen infected by *Haemoproteus spp.* (H&E). (7) Tissue section with hemozoin pigments (encircled) and few erythrocytes within the white pulp. (8) Tissue section showing white pulp hyperplasia and hemozoin pigments among red pulp.

In histopathological study of different tissues, schizonts with variable shapes and sizes were detected in endothelium of microcirculation of livers, lungs, kidneys and spleens in infected pigeons (Fig 9).

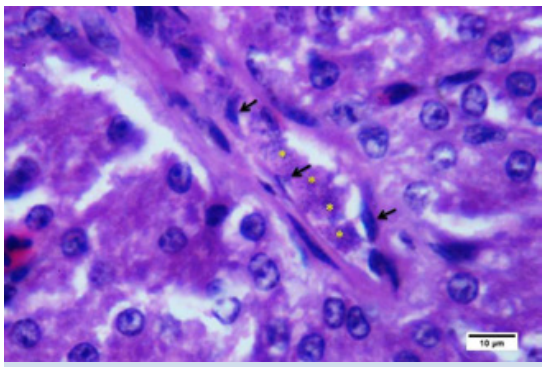

**Fig 9.** Schizonts of *Haemoproteus spp.* (stars) in the hepatic microcirculation of infected pigeon (short arrows show endothelial cells) (H&E).

**Table 4.** Frequency of histopathological lesions found in different tissues (kidney, lung and spleen) of pigeons infected by *Haemoproteus spp.*

| Tissue | Histopathological lesions | Prevalence (%) |
|---|---|---|
| **Kidney** | Multifocal non suppurative interstitial nephritis | 11 (84.6) |
| | Suppurative interstitial nephritis | 1 (7.7) |
| | Amyloidosis | 1 (7.7) |
| | Total | 13 (100) |
| **Lung** | Pneumonoconiosis | 8 (100) |
| | Total | 8 (100) |
| **Spleen** | Pigmentation | 12 (50) |
| | White pulp hyperplasia | 12 (50) |
| | Total | 100 |

## DISCUSSION

Throughout the world, the prevalence of *H. columbae* in pigeons in different geographical locations varies from 14 to 100 %. It has been reported to be 65.8 % infection in San Juan, Puerto Rico (Mclaughlin 1968), 70.4 % in Izmir, Turkey (Tolgay & Cesitli, 1972), 76.5 % in Kampala, Uganda (Dranzoa et al., 1999), 80 % from Sebele (Mushi et al., 2000), 57 % in wild pigeons of Ankara (Gicik & Arslan 2001), 37 % in domestic pigeons of Morogoro municipality of Tanzania (Msoffe et al., 2010) and 17.47 % from Ganbad, Golestan Province of North Iran (Youssefi et al., 2010). Most of the current data on haemosporidians, their distribution, vectors, parasite- host interactions and seasonality of infections have been collected primarily by microscopy (Atkinson & van Ripper 1991; Valkiūnas 2005). New molecular techniques have been considered to improve the detection of vector-borne blood parasites (Bensch et al., 2000; Ricklefs & Fallon 2002; Waldenstorm et al., 2004). Based on the results of the present study 34.2% of pigeons were infected with *Haemoproteus spp.* macro and microgametocytes by microscopic examination while, semi-nested PCR method detected 63.8%. Tavassoli et al., (2018) examined 93 blood samples from Iranian pigeons for *Haemoproteus spp.* In their study, 13 (13.97%) samples were positive in stained blood smears for *Haemoproteus spp.* and 27 (24.73%) were positive by PCR. Their results also revealed that PCR had higher sensitivity in detecting *Haemoproteus spp.* in pigeons. According to some studies, and based on the results of the present study, PCR tests were many-fold better than microscopy for detecting chronic blood parasite infections (Jarvi et al., 2002; Durrant et al., 2006). It has recently been shown that relative to microscopy methods, the PCR- based molecular methods generally provide higher estimates for *Haemoproteus spp.* (Garamszegi 2010).

It is known that after an initial acute phase of infection by blood parasites, the host develops chronic, low intensity parasitemia that is regulated by host cellular and humoral immunity. Whereas high-intensity acute parasitemias are typically easy to monitor and quantify by inspecting blood smears (Valkiūnas et al., 2008), the use of microscopy for diagnosing chronic, low-intensity infections may considerably underestimate parasite prevalence (Jarvi et al., 2002). Our results support conclusions of previous studies about insufficient sensitivity of microscopy when parasitemia is low. The low numbers of *Haemoproteus spp.* from microscopy samples may be explained mainly by difficulties to detect the patent infections of this haemosporidian genus during examining of blood smears of exceptionally light infections when just a few parasites are present in samples.

It is important to emphasize that, in most cases, the identification of haemosporidian species occurs through features observed by microscopy, such as the erythrocytic stages, including the length, width, area, size and number of hemozoin granules (Garnham 1966; Valkiūnas 2005; Martinsen et al., 2006). Therefore, this technique is still of great importance for the diagnosis of haemosporidian and should continue to be used together with molecular analyses.

In the present study histopathological changes of different tissues of naturally infected pigeons were investigated. Because schizogony does not occur in erythrocytes, infections cannot be experimentally transmitted by blood transfer and tissue transmission attempts rarely have been successful (Bierer et al., 1959). Host specificity, difficulties associated with experimentally infecting birds, limiting the number of feasible experimental models and few identified vectors also have restricted laboratory studies on the adverse effects of these parasites. Therefore, similar to our study most of the research about *Haemoproteus spp.* are based on natural infections. Histopathological lesions associated with *Haemoproteus* species occur in the pre-erythrocytic stages, which result from the formation of schizonts that can occur in a variety of cell types, and their presence causes tissue damage that may lead to the death of birds (Atkinson et al., 1986, 1988; Cardona et al., 2002; Donovan et al., 2008; Cannell et al., 2013). The following changes have been reported as related to *Haemoproteus spp.* infection: splenomegaly, hepatomegaly, multifocal hemorrhages in liver and spleen, necrosis (hepatocellular, liver, splenic, and cardiac), deposition of pigment (in Kupffer cells, hepatocytes, and macrophages of the lung and spleen), tissue displacement and inflammation (in the spleen, liver, lungs, and heart), and the presence of megaloschizonts in the liver and spleen, which were surrounded by a hemorrhagic inflammatory infiltrate composed of macrophages, heterophils, giant cells, and red blood cells (Atkinson et al., 1988; Peirce et al., 2004; Donovan et al., 2008; Cannell et al., 2013). Therefore, these results indicate that the histopathological changes found in this study may be related to *Haemoproteus spp.* But there were some differences in our study. Our main difference is the absence of megaloschizonts. Numerous uninucle-

ar merozoites, which are asexual stages of spreading with the vertebrate host, develop in exoerythrocytic meronts. The latter develop mainly in the endothelial cells and probably in fixed macrophages, while in some species the meronts mature in myofibroblasts. There are several generations of the exoerythrocytic development, during which the parasite gradually adjusts to the host. In the present study the meronts most frequently were found in livers of infected pigeons and less often in spleens, lungs, kidneys and hearts and they were variable in shape and size. Although most of the investigations have shown that the lungs have the high level of schizonts. Also, in most of the studies megaloschizonts of *Hamoproteus* have been detected while none of the infected pigeons in our study showed megaloschizonts. Albeit it should be mentioned that Valkiūnas (2015) believed that some species like *H. handai* and *H. mansoni* (syn. *H. meleagridis*) are able to produce huge meronts in the endothelial cells of capillaries, in myofibroblasts of the skeletal musculature, in the heart muscle, and sometimes in other muscular organs. Earle et al., (1993) suggested that all species of *Haemoproteus* are probably capable of forming schizonts in a variety of tissue and that the number of different tissues containing schizonts depends on the density of infection. In Dey et al., (2010) study comma shaped schizonts of *Haemoproteus spp.* were found in liver parenchyma accompanied with reactive cells. In their study the schizont like structures was seen in liver parenchyma which was surrounded by neutrophils. Hepatic cords were found to disappear in the affected area. The morphology of the schizonts identified here as those of *H. columbae* resemble those described in the literature by several authors including Baker (1966). However, Earle et al., (1993) described a range of morphological forms including megaloschizonts, from what was claimed to be *H. columbae* from a Bleeding-heart Dove in South Africa. As discussed by Lederer et al., (2002), the host from which Earle et al., (1993) described the schizonts is not endemic to Africa, and no such forms have been described from indigenous columbiform hosts. Thus, the conclusions were drawn by Earle et al., (1993) should be interpreted with caution as other parasites may have been involved. Although large schizonts have been recorded, the only true megaloschizonts confirmed for a species of *Haemoproteus* based on controlled experimental studies are for *H. meleagridis* in turkeys (Atkinson et al., 1986). The status of schizonts in psittacid tissues is unclear. Large megaloschizonts described from tissues of imported psittacines in Europe were initially thought to be an aberrant form of *Leucocytozoon*, even though no blood forms were ever observed (Walker & Garnham 1972; Peirce and Bevan 1977). However, subsequent studies suggested that these large multilocular schizonts were in fact *Besnoitia spp.* (Bennett et al., 1993; Peirce 1993). Schizonts with similar morphology have been observed in Pied Currawongs (Lederer et al., 2002). Overall, based on the results of the present study it seems that we should not expect megaloschizonts in pigeons infected with *Haemoproteus*.

In the present study focal lymphocytic aggregates, pigmentation, cell swelling and lipidosis were the histopathological findings in the liver. Nermean et al., (2016) claimed that liver histopathological changes of pigeons infected with *H. columbae* revealed a granuloma-like round cell infiltration formed mainly of small lymphocytes, many plasma cells, and schizonts of the parasite in and around the granuloma. As a rule, granuloma refers to a mass consisting mainly macrophages and its related cells (epitheloid and multinucleated giant cells), therefore it seems that in fact, the intended granuloma in their study was lymphocytic aggregates. Histopathological findings of the present study revealed pigmentation of liver, spleen, kidney, and lungs. The hem groups released from the digestion of the hemoglobin of infected red blood cells are aggregated into an insoluble material called hemozoin (Pagola et al. 2000). The α-hematin (ferriprotoporphyrin IX), which is toxic to the parasite, is released during hemoglobin digestion. However, and most possibly as a protection strategy, the parasite transforms α-hematin into hemozoin (chemically identical to β-hematin), a molecule with paramagnetic properties (Orjih 2001). In the present investigation, histopathological analysis of kidneys showed multifocal non-suppurative interstitial nephritis. Blockage of vessels, destruction of renal tubules and nephritis (consisting of granulocytes, macrophages and lymphocytes) were reported by Peirce et al., (2004) as histopathologic findings of *Haemoproteus spp.* infection. White pulp hyperplasia and pigmentation were the main histopathological changes in spleens of the infected pigeons of the present study. It should be mentioned that Tostes et al., (2015) reported alterations such as disorganization, intense congestion of the red pulp, hyperplasia, and hypertrophy of macrophages, brownish pigmentation, and splenic cords transformation into fibrous bands in caracaras infected with *Haemoproteus spp.* In the present study heart

of one infected pigeon showed mild lymphocytic myocarditis. In the study of Tostes et al., (2015) there were signs of myocardial and pericardial congestion in tissue sections of the hearts of infected caracaras. Overall, based on the molecular method 63.8% of pigeons were infected with *Haemoproteus spp.*. By comparing the pathological lesions of the present study with the results of other investigations, some similarities and differences were found. The differences may be related to different species of parasite, different hosts or even misdiagnosis of lesions. Focal lymphocytic aggregates, pigmentation and cell swelling were the main histopathological lesions in infected livers. Multifocal non- suppurative interstitial nephritis, pigmentation and splenic white pulp hyperplasia were also seen in the infected pigeons. Mild lymphocytic myocarditis in the heart of one pigeon was the other finding. No histopathological changes were seen in brain, intestine, and pancreas. Schizonts with variable shapes and sizes were detected in infected livers, lungs, kidneys, and spleens but megaloschizonts were not found. The main finding of this study was the absence of megaloschizonts of parasite in different infected tissues.

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## CONFLICT OF INTEREST

None declared.

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