Leucocytozoon spp. infection in Accipitriformes birds in Iran

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Abstract *Leucocytozoon* spp. (Haemosporida, Leucocytozoidae) are vector-borne parasites of various birds. *Leucocytozoon* can infect different reticuloendothelial tissues and blood cells of birds. In this study peripheral blood samples were collected from Accipitriformes birds [three marsh harriers (*Circus aeruginosus*) and one tawny eagle (*Aquila rapax*)] in one birds' garden in Iran. Blood films were observed for identification of hemoparasites. All samples were infected by different *Leucocytozoon* species. All of the observed species were first reported in Iran in Accipitriformes birds which one of them was described as a new species.

Keywords *Leucocytozoon* · *Circus* · *Aquila* · Accipitriformes birds · Iran

Introduction

The taxonomic review of Falconiformes and Accipitriformes birds is challenging (Christidis and Boles 2008). In traditional taxonomy of Falconiformes, two families the Accipitridae and Falconidae were accepted (Livezey and

Zusi 2007; Christidis and Boles 2008), but Ferguson-Lees et al. (2001) split the conventional Falconiformes into several orders, Pandionidae and Accipitridae were placed in the Accipitriformes, Falconidae in the Falconiformes and Sagittariidae into the Sagittariiformes and the Cathartidae were transferred to the Ciconiiformes. *Pandion*, *Hamirostra*, *Aviceda*, *Accipiter*, *Circus*, *Butastur*, *Aquila* and *Hieraetus* are some genera in Accipitriformes order (Christidis and Boles 2008).

Leucocytozoon spp., Family Leucocytozoidae, are haemosporidian parasites of numerous orders of birds. There are two different phases in *Leucocytozoon* life cycle; sexual phase in blood sucking simuliid flies (Simuliidae)—or biting midges (Ceratopogonidae) in *L. caulleryi*- and asexual phase in bird's host. In birds the parasite can be detected in different tissues as exoerythrocytic and erythrocytic phases. Sporozoites can develop only in the hepatocytes and can be detected in the birds for several days. The exoerythrocytic merogony occurs in hepatocytes, macrophages and other reticuloendothelial cells, including endothelial cell of capillary endothelium. The sporozoites of *Leucocytozoon* are the form of the parasite that can be injected by simuliid flies into bird's host. Gametocytes which develop in both roundish and fusiform host cells can be infective for diptera vector. Sporogony takes place in midgut of Simuliid flies. During sporogony; gametes develop and fertilize then produced sporozoites which have one rounded end and one pointed end can enter in the salivary glands of the vector (Valkiunas 2005).

Leucocytozoon spp. are usually nonpathogenic but some mortalities have been reported in ducks and geese. It can cause disruption of skeletal and cardiac muscles and liver damage is usually acute and severe. Haemorrhage and necrosis are observed in infected liver and gametocytes of the parasite can be found in peripheral blood films. Clinical

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signs can include anorexia, weakness, haemoglobinuria, depression and dehydration. Chloroquine, primaquine or pyrimethamine may be used for the *Leucocytozoon* treatment and exposure to biting insects should be prevented (Doneley 2010).

Birds' gardens in Iran are the places that municipal authorities design in some cities for tourism attraction. There are different species of birds which live close to each other in different parts of the gardens. In this environment, some diseases such as arthropod-borne diseases can be transmitted easily.

The aim of this study is to diagnose the haemosporidian parasites of Accipitriformes birds in one birds' garden in Iran.

Materials and methods

The samples were collected from a birds' garden near Tehran, the capital of Iran. The authorities of the birds' garden selected some birds and allowed us to collect just one peripheral blood film of each selected bird. In Accipitriformes birds in the garden, samples were taken from three marsh harriers (*Circus aeruginosus*) and one tawny eagle (*Aquila rapax*). The blood films were transferred to parasitology laboratory, fixed by methanol and stained by Giemsa. The stained blood films were observed under light microscopy for observation and diagnosis of hemoparasites.

Results

All of the taken blood films had been infected by different *Leucocytozoon* spp (Figs. 1, 2, 3). *L.toddi* group macrogametocytes and microgametocytes were observed in one *C.aeruginosus*, *L.nycticorax* in one *C.aeruginosus* and one *A.rapax*. One new species of *Leucocytozoon*, *L.persiensis* (n.sp.) was observed in one *C.aeruginosus*. The sizes of observed *Leucocytozoon* spp. microgametocytes and macrogametocytes were described.

The remark characteristics of *L.persiensis* (n.sp.) are; nucleus of host cell is not observed in macrogametocytes and microgametocytes both in roundish and fusiform host cells (Fig. 3). The fusiform host cell macrogametocytes and microgametocytes are wavy and irregular shaped and one end (7–10 μm) of fusiform host cell macrogametocytes and microgametocytes is wider than another end (2–3 μm), macrogametocyte in roundish host cell is $9 \times 10 \mu\text{m}$ and its nucleus is $2 \times 3 \mu\text{m}$, microgametocyte in roundish host cell is $16 \times 9 \mu\text{m}$ and its nucleus is not visible, macrogametocytes in fusiform host cell are $22\text{--}30 \times 5\text{--}6 \mu\text{m}$ and

Fig. 1 *Leucocytozoon toddi* group. nhc nucleus of host cell, np nucleus of parasite. **A** Macrogametocyte in fusiform host cell, $\times 300$, **B** macrogametocyte in fusiform host cell, $\times 100$, **C** Macrogametocyte in roundish host cell, $\times 200$, **D** microgametocyte in fusiform host cell, $\times 200$, **E** macrogametocyte in fusiform host cell, $\times 300$, **F** macrogametocyte in fusiform host cell, $\times 200$. Stained by Giemsa

their nucleuses are $4\text{--}5 \times 2\text{--}4 \mu\text{m}$, macrogametocytes in fusiform host cell are $26 \times 6\text{--}10 \mu\text{m}$ (Fig. 3C, D and F).

In diff quick: *C.aeruginosus* leukocytes were counted in 3 different samples were: lymphocyte (39, 31, 26%), heterophil (61, 56, 71%), eosinophil (0, 4, 3%) and monocyte (0, 9, 0%). In *A.rapax* sample lymphocyte was 43%, heterophil 44%, eosinophil 12%, monocyte 1%. There were also some abnormalities in leucocytes morphology which will be discussed later.

Discussion

After the first records of *L.todii* in *Accipiter nisus*, and *Accipiter bandius*, some differences were observed in microgametocytes and macrogametocytes of the same and different vertebrate hosts, therefore it was recommended that for *L.todii*, identification the form of infected host cells and the form and location of the parasite nucleus are important (Greiner and Kocan 1977; Valkiunas and Iezhova 1990). Valkiunas et al. (2010) claimed that there are different species of *L.todii* in molecular examination and their gametocytes morphologies are also different. According to that study, *L.mathisi*, *L.buteonis* and *L.todii* were placed in a *Leucocytozoon toddii* group. In this study, differences were observed in macrogametocytes in fusiform host cells and all of them (same or different species) were reported as *L.todii* group because the molecular examination was not possible in this study. *L.todii* group in

Fig. 2 *Leucocytozoon nycticoraxi*, *nhc* nucleus of host cell, *np* nucleus of parasite, *a* cytoplasmic accessory. **A** Macrogametocyte in fusiform host cell, bilobed eosinophil nucleus (black arrow) with decreased cytoplasmic granules, $\times 100$, **B** macrogametocyte in fusiform host cell, $\times 100$, **C** macrogametocyte in fusiform host cell, $\times 150$, note the different shapes and lengths of cytoplasmic accessories, **D** macrogametocyte in fusiform host cell, $\times 200$, **E**, **F** macrogametocyte in fusiform host cell, $\times 50$, note the different shapes and lengths of cytoplasmic accessories, **G** microgametocyte in fusiform host cell, $\times 70$, **H** macrogametocyte in fusiform host cell, $\times 100$, **I** macrogametocyte in fusiform host cell, $\times 300$. **A–G** from *C.aeruginosus* and **H** and **I** from *A.rapax* blood films. Stained by Giemsa

roundish host cells were rarely observed as previous studies (Peirce and Marquiss 1983, Valkiunas 1989). The sizes of observed *L.todii* group parasites in this study were in the range of previously reported studies.

L.todii group parasites have been reported in Ethiopian, oriental, neotropical zoogeographical regions (Valkiunas

Fig. 3 *Leucocytozoon persiensis* (n.sp.), *np* nucleus of parasite. **A** Macrogametocyte in roundish host cell, $\times 500$, **B** macrogametocyte in roundish host cell, $\times 250$, **C** macrogametocyte in fusiform host cell, $\times 300$, **D** macrogametocyte in fusiform host cell, $\times 200$, **E** microgametocyte in roundish host cell, $\times 500$, **F** microgametocyte in fusiform host cell, $\times 200$. Stained by Giemsa

2005) in Accipitriformes and Falconiformes birds. In Congo (Greiner and Kocan 1977), Kazakhstan, the prevalence of *L.todii* in *Accipiter nisus* was more than 94% and in genus *Circus* was more than 73% (Valkiunas 1989), *L.todii* has been reported in Accipitridae birds in India (Nandi 1999), in Iraq *L.todii* has been observed in 15.4% of *Buteo buteo vulpinus*, 12.5% of *Circus aueruginosus* and 8.7% of *Faico tinnunculus* (Jasim 2006) and 8.8% of *Himantopus himantopus* (Mohammad and Al-Moussavi 2013). In Czech Republic the prevalence of *Leucocytozoon* spp. were 1.9–100% in *Buteo buteo* (Svobodova et al. 2014) and in another study in Czech republic *L.todii* group has been reported in 80% of adult and 13.6% of nestling of *Accipiter gentilis* (Hanel et al. 2015).

Leucocytozoon nycticoraxi (Shamsuddin and Mohammad 1980) has been reported in Iraq in *Nycticorax nycticorax* (Ciconiiformes). The analysis of gametocytes of *L.nycticoraxi* indicated that it can be synonym of *L.leboeufi* (Valkiunas 2005). In this study, more shapes of Macrogametocytes were observed. The most interesting thing in observed *L.nycticoraxi* was the different lengths and widths of microgametocyte cytoplasmic processes, described as “long” ($15\text{--}16 \times 1\text{--}7 \mu\text{m}$) and “short” ($4\text{--}7 \times 1\text{--}4 \mu\text{m}$) ones. In Shamsuddin and Mohammad, 1980, the remnants of cytoplasmic processes were not observed. In this study Fig. 24 and 5 can be compared; In Fig. 2D and E the

cytoplasmic processes are not detectable among reticulocytes but in Fig. 2E two different (long and short) cytoplasmic processes can be detectable which were lined in Fig. 2F. Therefore, cytoplasmic processes in *L.nycticoraxi* macrogametocytes present different lengths (Fig. 2C and E).

In addition, *L.nycticoraxi* was observed in *C.aeruginosus* and *A.rapax* in this study and in comparison with previous study in *N.nycticorax* it can be concluded that *Leucocytozoon* species are not host-specific parasites and this characteristic in some places such as birds' gardens become more important because an infected vector can infect various birds one after another as “domino effect”.

All of these infected birds were depressed and were not as sharp as prey birds. All of these reported species of *Leucocytozoon* were first records in Accipitriformes birds in Iran and *L.persiensis* was first described in *C.aeruginosus* around the world.

There were not a lot of information about changes of reticulocyte and leucocyte morphologies in haemosporidian infected Accipitriformes birds. Most of the observed abnormalities were decreased cytoplasmic granules in eosinophils and heterophils. The band cells and cytoplasmic vacuolation of heterophils were also observed as toxic changes. Reactive lymphocytes are antigenic stimulated lymphocytes and in these infected samples were observed as lymphocytes with increased in cytoplasmic basophilia and peripheral cytoplasmic vacuoles. All of these changes can be detectable in inflammatory conditions of birds (Clark et al. 2009). Increase in lysed reticulocytes and anisocytosis as a sign of regenerative anemia were also observed.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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