

A Study on the Presence of Leucocytozoonosis in Wild Birds of Burdur District

Özlem ÖZMEN*, Mehmet HALIGÜR

Department of Pathology, Faculty of Veterinary Medicine, Akdeniz University, 15100 Burdur - TURKEY

Bayram Ali YUKARI

Department of Parasitology, Faculty of Veterinary Medicine, Akdeniz University, 15100 Burdur - TURKEY

Received: 22.10.2004

Abstract: From 2000 to 2004, 53 wild birds (16 pigeons, 16 quails, 10 pheasants, 4 partridges, 3 stock doves, and 1 ibis, peacock, owl chick and falcon) were examined grossly and microscopically at the Department of Pathology, Faculty of Veterinary Medicine, University of Akdeniz. Thirty-seven of these birds were dead. Blood samples were taken from the 16 live birds before euthanasia. Tissue samples were also taken for histopathological examination during the necropsies. *Leucocytozoon marchouxi* was diagnosed in 5 pigeons during the microscopic examinations of blood samples and tissue sections, whereas 1 owl chick died from acute severe *L. ziemmani* infection.

Key Words: Wild birds, Leucocytozoon, blood parasite

Burdur'da Yabani Kuşlarda Leukositozoonozis'in Varlığı Üzerine Bir Çalışma

Özet: Akdeniz Üniversitesi, Burdur Veteriner Fakültesi, Patoloji Anabilim Dalı'nda 2000-2004 yılları arasında 53 yabani kuşun (16 güvercin, 16 bildircin, 10 sülün, 4 keklük, 3 üveyik ve birer adet ibis, tavus kuşu, baykuş yavrusu ve şahin) makroskobik ve mikroskobik incelenmesi yapıldı. Bunlardan 37 tanesi ölü olarak getirildi. Canlı olan 16 kuştan ötenazi öncesi kan örnekleri alındı. Nekropsi sırasında dokulardan histopatolojik muayene için örnekler toplandı. Kan örneklerinin ve doku kesitlerinin histopatolojik yoklamasında beş güvercinde *L. marchouxi* teşhis edildi. Bir baykuş yavrusunun ölüm sebebi akut, şiddetli *L. ziemmani* enfeksiyonuydu.

Anahtar Sözcükler: Yabani kuş, Leucocytozoon, kan paraziti

Introduction

Four genera (Plasmodium, Haemoproteus, Leucocytozoon and Trypanosoma) of blood parasite are well known as avian haematoprotezoa observed in a variety of bird species (1). Most species of Haemoproteus and Leucocytozoon are relatively host-specific and restricted to bird species of the same family. This is in contrast to species of Plasmodium, which have a much broader host specific and occur in several avian families by changing their character (2-7). Leucocytozoonosis is a parasitic disease of anseriformes, turkeys, raptors, wild birds and columbiformes. Leucocytozoon sp. are named after the species in which they are found, for example, *L. simondi* in anseriformes, *L. smithi* in turkeys, *L.*

marchouxi in columbiformes, *L. toddi* in falconiformes, and *L. ziemmani* in owls (2-4).

Leucocytozoonosis is a protozoal disease of birds that affects the blood and tissue cells of internal organs (1). This parasite was assigned to the suborder Haemospororina of the phylum Apicomplexa (8). Leucocytozoon sp. use Simuliidae (black flies) as vectors (9). The life cycle includes reproduction by sporogony in insects with schizogony (merogony) in tissue cells, and gametogony in erythrocytes or leukocytes (1). Initial development occurs in the liver and spleen, followed by the development of unpigmented gametocytes in white or red blood cells, depending upon the species (9). Infected host cells are distorted beyond recognition.

Leucocytozoon has a seasonal incidence in the wild, with parasitaemia being highest in the spring (1,9).

Clinical signs vary with the age and condition of the host. Young birds manifest inappetence, weakness, listlessness, dyspnoea, and sometimes death within 24 h. Signs in adults appear less abruptly and consist of listlessness and a low mortality rate (1,10). Some other pathologic manifestations of the disease are anaemia, leukocytosis, splenomegaly, and liver degeneration and hypertrophy (1).

The parasites have an almost worldwide distribution and occur in a variety of species, including ducks, geese, turkeys, domestic fowl, game birds, owls and other birds of prey, numerous passerines and parakeets (excluding budgerigars). Although there is a very limited number of studies on blood parasites of avian species (2,11,12), there is no report describing the presence of Leucocytozoon sp. in Turkey. The aim of this study is to report the leucocytozoonosis and to examine pathological findings in this illness in wild bird species in Burdur district.

Materials and Methods

Fifty-three different kinds of wild bird were examined macroscopically and histopathologically between 2000 and 2004 at the Department of Pathology. The most common reason for the wild birds' being brought to the faculty was traumatic injuries caused by illegal hunting. Some others were brought by their owners who kept the wild bird in their houses. The main causes of mortality in these birds were several diseases (aspergillosis, Newcastle, Marek ect.). The others were accidentally found sick or injured.

Thirty-seven birds (10 pigeons, 16 quails, 5 pheasants, 2 partridges, 2 stock doves, 1 peacock and 1 owl chick) were dead, while 16 birds (6 pigeons, 5 pheasants, 2 partridges, 1 stock dove, 1 ibis and 1 falcon) were alive but in very bad condition. Due to the poor prognosis, these living birds were euthanised after bleeding. Prepared blood smears were sent to Department of Parasitology for the diagnosis of blood parasites. The smears were fixed in methanol for 5 min and stained with Giemsa solution for 45 min and examined under the immersion objective. Necropsy was performed on all dead and euthanised birds and tissue samples were taken from all organs. Tissues were fixed

in 10% neutral-buffered formalin and routinely processed and embedded into paraffin for histopathological examination. Tissues were sectioned at 5 μ and stained with haematoxylin and eosin (HE). In some selected cases periodic acid Schiff (PAS) and May-Grunwald Giemsa method (13) for tissue sectioning were also used.

Results

Thirty-seven out of the 53 birds were dead. Twenty-nine birds (14 pigeons, 9 quails, 3 pheasants, 2 partridges, 1 stock dove) were brought in by staff of the Environment and Forestry Directorship of Burdur. They collected the birds from hunters engaged in illegal hunting. All of these birds had bone fractures of the wings and legs due to shooting. Severe wounds were observed on different parts of the body, especially localised on the head. Due to severe injuries, secondary bacterial infections and pus formations were common findings in the live birds.

None of the live birds showed clinical symptoms related to leucocytozoonosis. During the examination of blood smears, elliptical gametocytes were observed in the red blood cells. Approximately half of the parasite's perimeter adhered to the host cell nucleus. The parasite's nucleus was elongated, with a clear nucleolus close to the nuclear membrane. The host cell nucleus was wider at the ends and narrow at the centre. According to these characteristics, the parasite was morphologically identified as *L. marchouxi* in 3 pigeons (Figure 1). At necropsy there were no findings associated with the disease. Additionally, in 2 dead pigeons the parasites were also observed in blood vessels histopathologically because of the lack of blood smears and schizonts were observed in the cells of internal organs.

A 1-month-old dead owl chick was brought in for necropsy by the owner. He stated that he had found the bird in his garden after it had fallen from a nest 3 weeks previously. He had put the bird in a cage and started to feed it. Clinically the bird had seemed healthy until 2 days before death. According to the owner, the bird had shown the signs of inappetence, dullness and dyspnoea before death. During the necropsy fibrinous perihepatitis and swollen kidneys together with pale serosal membranes were the prominent findings (Figure 2). At histopathological examination, numerous Leucocytozoon

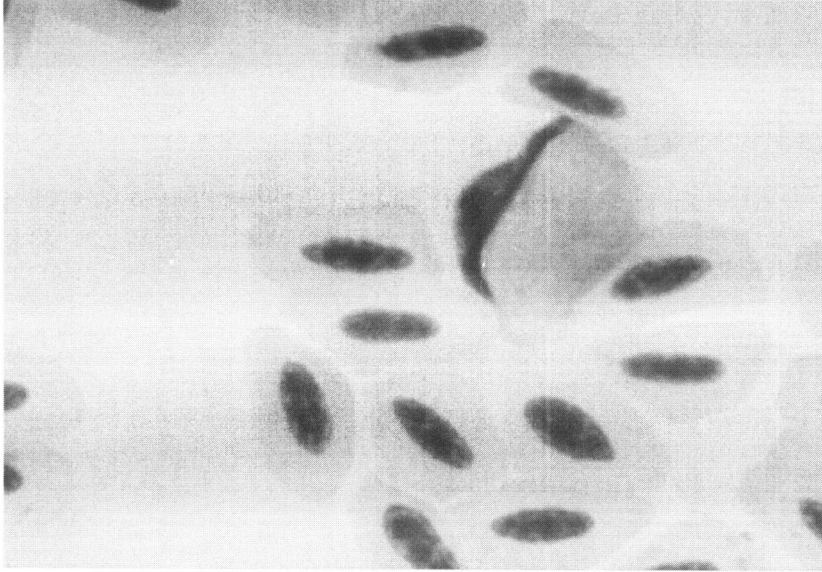


Figure 1. *Leucocytozoon marchouxi*, in a blood smear, pigeon, Giemsa stain, immersion oil.

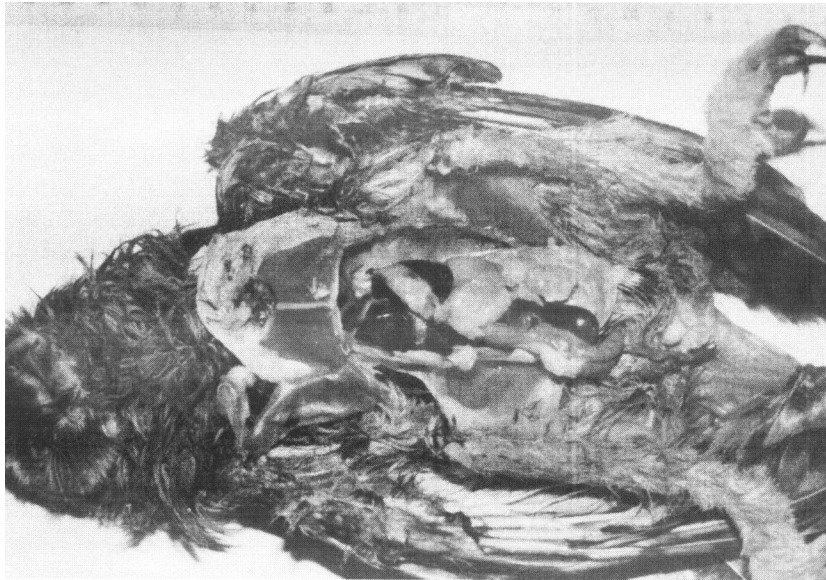


Figure 2. Fibrinous perihepatitis in an owl chick.

developmental stages (micro- and macrogametocytes) were seen in many blood vessels of the organs examined. Oval to irregular shape gametocytes were observed in vessels (Figures 3 and 4). More than half of the parasite's perimeter adhered to the host cell nucleus. Pieces of the host cell cytoplasm were often observed. There was no inflammatory reaction around the vessels. The agents

were seen in all of the vessels. They were numerous in the lung, kidney and liver vessels but no schizonts were seen in the cells of these organs. Small necrotic foci were observed in the liver and kidney. It was easy to see the parasite's developmental stages in vessels in HE stained slides. *L. ziemanni* was PAS negative and stained dark blue in May-Grunwald Giemsa stained sections.

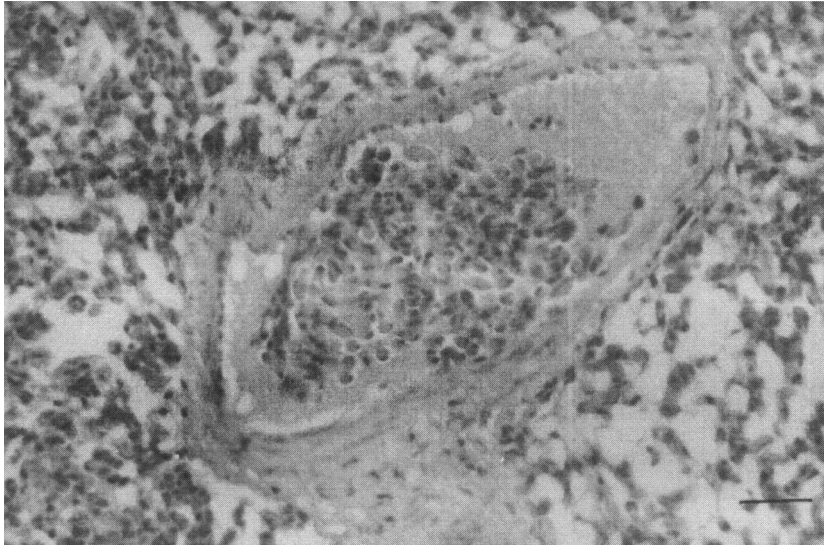


Figure 3. Numerous *L. ziemanni* in a lung vessel, owl chick, H.E., Bar = 200 μ m.

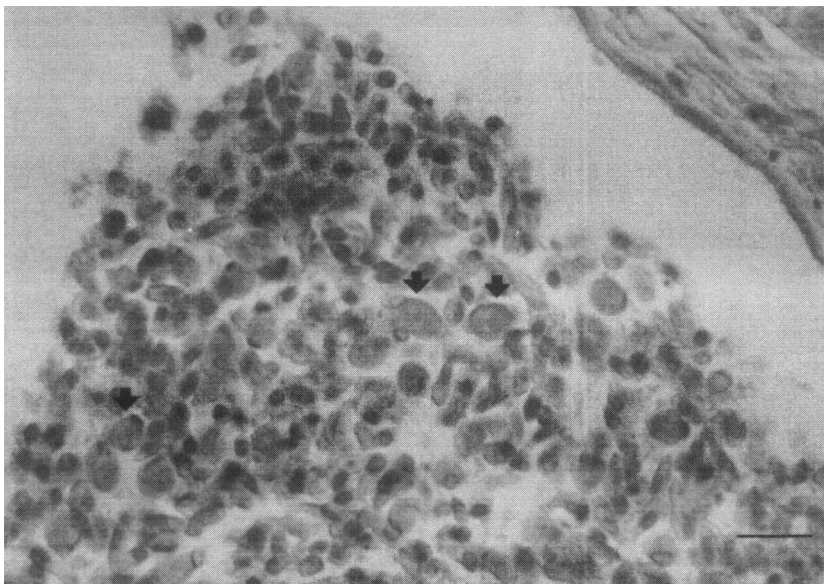


Figure 4. *L. ziemanni* in a liver vessel (arrows), owl chick, H.E., Bar = 50 μ m.

Discussion

Leucocytozoon sp. are arthropod-transmitted protozoa related to Plasmodium sp. Species of these protozoa appear to be widely distributed in many parts of the world (14). Despite the large number of bird species known to be susceptible to infection with Leucocytozoon, very few records are described and disease due to infection is rarely mentioned. Hemoprotozoa are

generally considered only slightly pathogenic to wild birds. However, some evidence exists that they can cause subclinical/debilitating effects and depress breeding rates, and thus their influence on the dynamics of natural bird populations should not be neglected (5). While the prevalence of leucocytozoonosis is lower than that of other haemoprotozoa in wild birds, it has a higher incidence in owls (6,7). In the present study,

Leucocytozoon sp. were observed in only 5 pigeons and 1 owl chick out of 53 birds. Although pigeons were asymptomatic carriers of the protozoon, the owl chick died from leucocytozoonosis.

Hemaprotozoa infection has been suggested to be species-specific; criteria for species designation include host range and gametocyte features, such as staining characteristics, size, nature, and extent of distortion of the host cell and altered shape and position of the host cell nucleus (1). In blood smears from 3 pigeons, rare, large, elongated, bluish, granular, intracytoplasmic *Leucocytozoon marchouxi* were observed. *L. ziemanni* was diagnosed in the owl chick because of the morphology of gametocytes and host specificity.

Leucocytozoon is the largest haemoparasite encountered in birds. The *Leucocytozoon* gametocyte does not contain refractile pigment and grossly distorts the host cell. Two forms are commonly encountered: an elongate form in which the host cell nucleus is pushed to the side, and the cytoplasm is long with tapering tails; and a round form in which the host cell nucleus is eccentrically located, and the cytoplasmic tails are obvious (10). The appearance of both round and elongated gametocytes is related to the pathogenicity of the *Leucocytozoon* strain, with the more pathogenic forms progressing to the elongate stage (1). Only the elongated form of the parasite was seen in pigeons in this study. Both elongated and round forms were observed in the owl chick, but elongated forms were numerous.

Leucocytozoon is occasionally pathogenic in young raptors, turkeys, and waterfowl, in which it may result in haemolytic anaemia and haemoglobinuria (10,16,18). Mortality may be high and in parakeets the parasite's presence in the cardiac muscle may cause heart failure. Following infection, high numbers of the parasite may be detected in the blood within 4 to 9 days. The parasite produces an antierythrocytic factor, which causes intravascular haemolysis and anaemia, and these remain the principal clinical signs. In this study all of the pigeons were adults and for that reason no clinical or gross findings associated with leucocytozoonosis were observed. The owl chick had severe gross findings such as anaemia, hepatomegaly, splenomegaly and swollen kidneys, but haemolytic anaemia and haemoglobinuria were not seen.

There were no schizonts in the cells of the internal organs examined.

Leucocytozoon transmission begins each year with a spring relapse in previously infected birds. Birds with circulating elongate gametocytes are infective, while those with circulating round gametocytes do not experience this relapse and are not infective. The circulating elongate gametocytes from the bird enter a blackfly during a blood meal. Sexual development takes place within the blackfly's digestive system and sporozoites enter the salivary glands of the blackfly, where they are injected into a susceptible avian host during a subsequent blood meal. In temperate zones the disease is seasonal, occurring when the insect carriers are active (1,10). The disease was diagnosed in June in the owl chick in the present study and the vectors were very active during this period. Because wetlands offering a suitable habitat for potential vectors are abundant in our province, they can be found in very crowded groups in Burdur.

Blood parasites are usually diagnosed in blood smears. *Leucocytozoon* infections are diagnosed by direct microscopic observation and identification of gametocytes in stained blood or schizonts in tissue sections (1-4,10). This study also showed that the best method for the diagnosis of blood parasites is examination of blood smears. However, if the birds are brought in dead it is possible to diagnose blood parasites by histopathological examination. The other possible method for the diagnosis of blood parasites in dead birds may be organ smears prepared before formalin fixation of the tissues.

In conclusion, this is the first report of the presence of *Leucocytozoon* sp. in wild birds in Turkey. Due to the suitable climate, vector black flies are very common throughout Turkey. Both wild and domestic avian species are susceptible to infection with *Leucocytozoon* sp. For that reason, leucocytozoonosis can be found in all avian species, including poultry.

Acknowledgement

The authors thank Akdeniz University Scientific Research Projects Unit for its support.

References

1. Springer, W.T.: Other blood and tissue protozoa. In: Calnek, B.W, H.J. Barnes, H.J. Beard, McDougald, Saif, Y.M., Eds. Diseases of poultry. Iowa State Press, Iowa.1997; 900-905.
2. Mimioglu, M., Göksu, K., Sayın, F.: Veteriner ve Tıbbi Protozooloji II. Ankara Üniv. Basımevi, Ankara. 1969; 121-130.
3. Tolgay, N: Evcil ve Yabani Kanatlıların Önemli Parazitleri. Ankara Üniv. Basımevi, Ankara. 1973; 872-880.
4. Soylsby, E.J.L.: Helminths, Arthropods and Protozoa of Domesticated Animals, Bailliere Tindall, London. 1982; 703-705.
5. Atkinson, C.T.: Host specificity and morphometric variation of *Haemoproteus meleagridis* Levine, 1961 (Protozoa: Haemosporina) in gallinaceous birds. Can. J. Zool., 1986; 64: 2634-2638.
6. Fallis, A.M., Desser, S.S., Kahn, R.A.: On species of Leucocytozoon. Adv. Parasitol., 1974; 12: 1-67.
7. Bennet, G.F., Whiteway, M., Woodworth-Lynas, C.B.: A host-parasite catalogue of the avian haematozoa . Occasional papers in biology, No.5, Memorial University of Newfoundland, 1982.
8. Levine, N.D., Corliss, J.O., Cox, F.E.G., Derroux, G., Grain, J., Honinberg, B.M., Leedale, G.F., Loeblich III, A.R., Lom, J., Lynn, D., Merinfeld, E.G., Page, F.C., Poljansky, G., Sprague, V., Vavra, J., Wallace, F.G.: A newly revised classification of the protozoa. J. Protozool., 1980; 27: 37-58.
9. Ritchie, B.W.: Identification of common blood parasites. In: Ritchie, B.W., Harrison G.J., Harrison, L.R. Eds. Avian Medicine: Principles and Application, Wingers Publishing Inc. Florida, 1994; 190-191.
10. Schmidt, E.R.: Pathology of cage birds. In: Rosskopf W.J., Woerpel, R.W., Eds. Disease of cage and aviary birds. Williams and Wilkins, Pennsylvania, 1996; 868-869.
11. Tolgay, N: Çeşitli kanatlıların Plasmodium, Haemoproteus ve Leucocytozoon enfeksiyonları üzerinde araştırmalar. Ankara Üniv. Vet. Fak. Derg., 1972; 19: 271-286.
12. Gıcık, Y., Arslan, M.Ö.: Blood parasites of wild pigeons in Ankara district. Turk J. Vet. Anim. Sci., 1999; 25: 169-172.
13. Luna, L. G.: Methods for Hematologic and Nuclear Elements. In: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, McGraw-Hill Book Company, New York. 1968; 121 and 158.
14. Jordan, F.T.W., Pattison, M.: Leucocytozoonosis. In: Poultry Disease, Saunders Company, London. 1999; 280.
15. Santos, N., Tome, P., Ferrand, N., Korpimaki, E.: *Leucocytozoon ziemanni* infection in a wild population of little owl *Athene noctua*. The Sixth Conference of the European Wildlife Disease Association, TSE and CWD Workshop, 8-12 September 2004, Uppsala, Sweden, pp 27.
16. Shurunlikov, P., Golemanky, V.: Plasmodium and Leucocytozoon (Sporozoa: Haemosporida) of wild Birds in Bulgaria. Acta Protozool., 2003; 42: 205-214.
17. Krone, O., Priemer, J., Streich, J., Sömmer, P., Langgemach, T., Lessow, O.: Haemosporida of birds of prey and owls from Germany. Acta Protozool., 2001; 40: 281-289.
18. Murata, K.: Prevalence of blood parasites in Japanese wild birds. J. Vet. Med. Sci., 2002; 64: 785-790.