NEU PRESS

REPVAS
RESEARCH & PRACTICE in VETERINARY & ANIMAL SCIENCE

Vol: 1 No: 1 Year: 2024 Research Article

Histopathological Investigation of Gastrointestinal System Parasites in Storks: Cathaemasia Hians

Özgür KANAT

Necmettin Erbakan University, Faculty of Veterinary Medicine, Department of Pathology, Ereğli, Konya, Türkiye
https://orcid.org/0000-0002-9747-5496

Article Info

Article History

Received: 01.11.2023
Accepted: 28.12.2023
Published: 31.01.2024

Keywords:
Cathaemasia Hians,
Gastrointestinal System,
Histopathology,
Stork,
Trematode.

Corresponding Author:
Özgür KANAT,
vetstorm@gmail.com

ABSTRACT

Intestinal parasites are commonly reported in free-ranging birds and play an important role in maintaining a healthy and balanced ecosystem and causing zoonotic diseases. The aim of this work was to reveal the presence and histological findings of digestive system parasites in storks, as well as the histopathological changes caused by C. hians isolated from storks. A total of four storks, an adult black stork and three adult white storks that were brought to the pathology laboratory were used in the study. Histopathological findings of the intestines of black and white storks were determined using hematoxylin and eosin staining. During necropsy, numerous nodules were observed in the serosa of the intestines of black stork. A parasitic infection was only observed along the oesophagus. Histopathologically, in the intestines of the black stork, degeneration, necrosis and desquamation, heterophil and mononuclear cell infiltrations, parasite sections and parasite eggs, ulcerated areas, and regeneration with fibrosis were detected. Histopathologically, no parasitic infections were found in the white storks' oesophagus or proventriculus. Degeneration, necrosis, and desquamation were observed in the intestinal epithelium of white storks. This study was the first to define histopathological changes in the intestines of storks and showed severe nodular inflammation caused by C. hians in Türkiye.

Citation: Kanat, Ö. (2024). Histopathological investigation of gastrointestinal system parasites in storks: Cathaemasia hians. *Research and Practice in Veterinary and Animal Science (REPVAS)*, 1(1), 9-18. https://doi.org/10.69990/repvas.2024.1.1.2

INTRODUCTION

Storks are members of the family Ciconiidae, which is part of the order Ciconiiformes, which comprises several families. This family contains 19 species of storks divided into six genera, many of which are migratory. The white stork (Ciconia ciconia Linnaeus, 1758), which is a Palearctic migrant (Girisgin et al., 2017), is a carnivore that consumes small animals, birds, amphibians, fish, and insects. The white stork lives in agricultural areas and is mostly associated with wetlands (Tylkowska et al., 2019). The black stork (Ciconia nigra Linnaeus, 1758), an endangered species of migratory waterbird (Karaska et al., 2002; Königova et al., 2015), feeds on frogs, salamanders, amphibians, fish, and mollusks found in swamps and slow-moving waters (Merino et al., 2001; Liptovszky et al., 2012). This species is one of the protected animals in our nation. Free-ranging birds play a vital role in maintaining a healthy and balanced ecosystem (Nasiri et al., 2019). Birds in zoological collections frequently carry various gastrointestinal parasites (Penczykowski et al., 2016; Moreno Manas et al., 2019). Because high levels of stress occasionally weaken the host's immune response and confinement increases the risk of inter- and intraspecific transmission, these parasites frequently cause disease in confined birds (Carrera-Jativa et al., 2020).

Numerous pathogenic parasites negatively impact humans, domestic animals, captive birds, and wildlife (including fish, birds, terrestrial mammals, and aquatic mammals) (Otegbade and Morenikeji, 2014). Both wild and domesticated birds are commonly infected by a variety of intestinal parasites that can be found in a variety of geological settings and climatic conditions, including protozoans, trematodes, cestodes, acanthocephalans, and nematodes. Because most birds serve as primary and/or secondary hosts for some parasites, this has an impact on the health of both animals and people (Girisgin et al.,2017; Nalubamba et al., 2015; Chaudhary and Pandey, 2020).

Helminths are classified as trematodes (flukes), cestodes (tapeworms), and nematodes (roundworms) (Muller, 2009). *Cathaemasia hians*, which became a Cathaemasiidae trematode, is a known parasite of storks (Ciconiformes). However, these species is rarely reported in Europe (Liptovszky et al., 2012). The gastrointestinal tracts of the white stork (Ciconia ciconia) and the black stork (Ciconia nigra) both have typical parasites of the Cathaemasia genus (Königova et al., 2015). Such trematodes have the ability to cause permanent changes in the gastrointestinal system tissues of their target hosts. Physiological digestion is directly affected by severe trematode infections. Therefore, histopathological changes are crucial for the digestive system of bird hosts. There are few studies describing how parasite infection causes histopathological abnormalities in the gastrointestinal system of wild birds. Additionally, wild birds can typically be captured at varied post-mortem times, which makes it difficult to perform specific histopathological analyses of organs (Königova et al., 2015). An in-depth and precise diagnosis is made possible by histopathological examination of organs or tissues. An understanding of pathogen interactions and their effects on the host organism can be gained by histopathological analysis (Soltysiak et al., 2014). Histopathological changes occurring during certain parasite invasions are critical for differential diagnosis and frequently confirm the presence of parasitic infections. The objective of this study was to reveal the presence and histological findings of digestive system parasites in storks as well as the histopathological changes caused by C. hians isolated from storks.

MATERIALS AND METHODS

In this study, a total of four storks were used: an adult black stork and three adult white storks brought to the Pathology Department of the Veterinary Faculty, Hatay Mustafa Kemal University, for necropsy between 2016 and 2021. All storks were necropsied, and their digestive systems were examined macroscopically and histopathologically. After fixation of tissues (especially digestive system tissues) received from all storks in 10% buffered formalin solution. The samples were routinely treated and embedded in paraffin. Sections were cut to 5 µm thickness and stained with hematoxylin-eosin (HE) (Luna, 1968; Dörtbudak et al., 2019; Colcimen et al.,2020) and examined under a light microscope. During systemic necropsy, 32 adult trematodes were collected from the oesophagus, and 34 nematodes were collected from the abdominal and thorax cavities of the black stork, particularly the air sacs. However, macroscopically, parasites were not observed in white storks. Trematodes and nematodes were fixed in 70% alcohol. Species of parasites were determined by Işık et al. (Işık et al., 2017) in the Department of Parasitology, Faculty of Veterinary Medicine, Selcuk University. However, the lesions and histopathological changes in the gastrointestinal tissues of infected storks were the focus of this investigation.

RESULTS

The nematodes found in the air sacs and abdominal and thoracic cavities during necropsy were *D. ciconiae* (Figure 1A), whereas the trematodes found along the oesophagus were *C. hians* (Figure 1C). Numerous whitish-colored nodules of hard consistency were observed in the serosa of the intestines of black stork. Other than the intestine, no other internal organs were found to have pathological lesions during necropsy (Figure 1B). The species of trematodes observed in the oesophagus were determined to be *Cathaemasia hians* by parasitological examination (Figure 1D).

Histopathologically, in the intestines of the black stork, degeneration, necrosis, and desquamation of the epithelium were observed, as were heterophil granulocyte and mononuclear cell infiltration in the propria, as well as various parasite formations and parasite eggs in both the intestinal lumen and the propria (Figure 2A-B). The ulcerated area formed because of the trematode passage into the muscular layer from the intestinal mucosa and was regenerated with connective tissue (Figure 2C). The parasites were particularly found in the muscular layer. Furthermore, parasite eggs were detected in the propria (Figure 2D). It was observed that the nodules observed macroscopically contained adult parasites and their eggs under the microscope (Figure 2E-F). No parasitic infections were observed in the oesophagus and proventriculus of white storks (Figure 3A). The proventriculus of the black stork showed epithelial degeneration, necrosis, desquamation, and mild mononuclear cell infiltration (Figure 3B). Degeneration, necrosis, and desquamation were observed in the intestinal epithelium of white storks, as well as mild cell infiltration and fibrosis in the propria (Figure 3C-D). Histopathologically, neither adult parasites nor parasite eggs were found in the white storks.

Figure 1. Macroscobic and parasitic examination. A) *Dicheilonema ciconiae* in the air sacs and thoracic cavity (arrows). B) Nodules in the intestines (arrow). C) Adult *Cathaemasia hians* in the oesophagus (arrow heads). D) *C. hians* through an optical stereoscope.

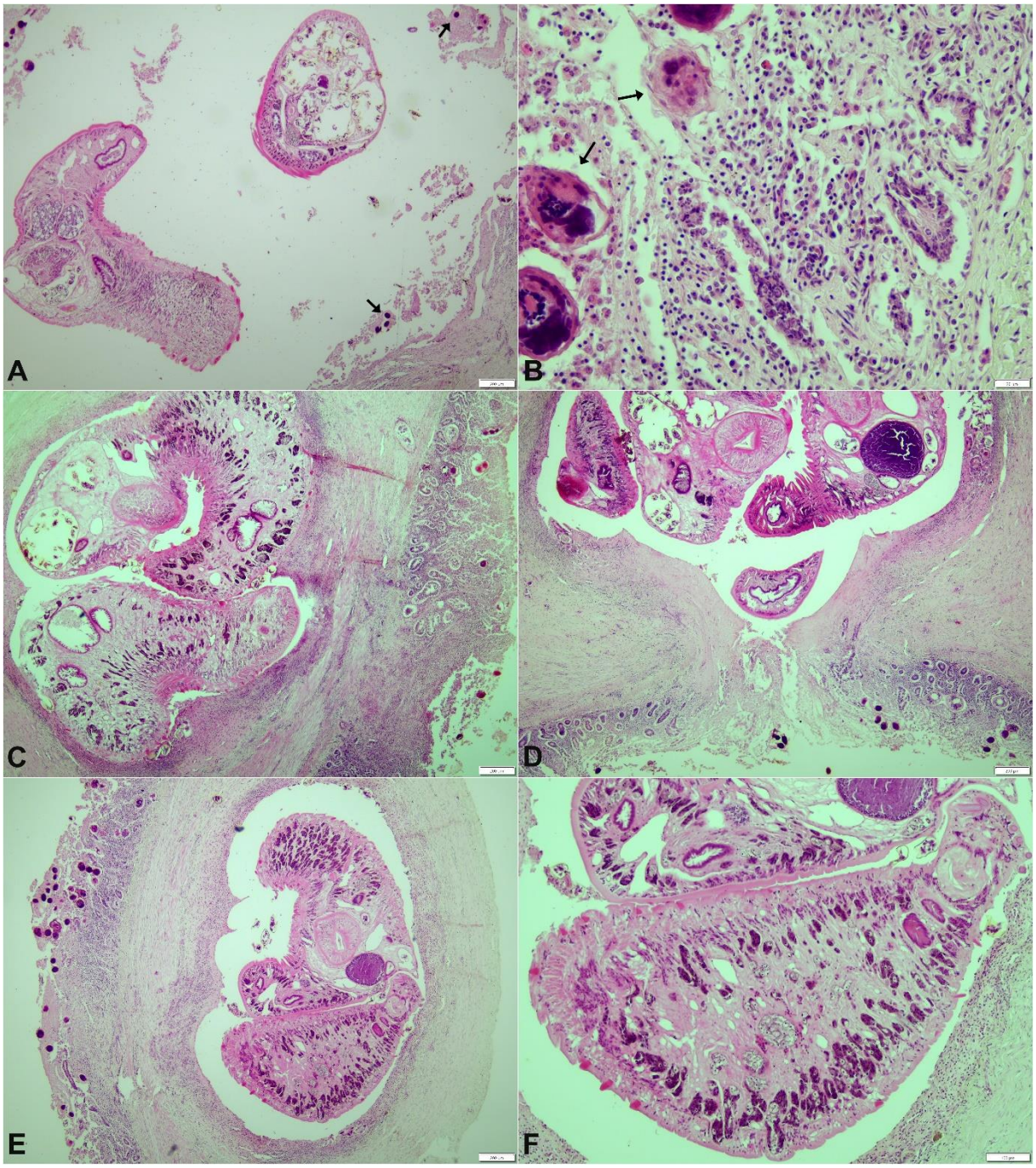

Figure 2. Microscobic findings of black stork. A) Adult trematodes and parasite eggs (arrows) in the intestinal lumen. B) Degeneration and desquamation in the epithelium, heterophil granulocyte and mononuclear cell infiltration, and parasite (arrows) in the propria. C) Regeneration with fibrosis in the mucosa and adult trematodes in the muscular layer. D) Ulcers and severity of adult and eggs of trematodes. E) Adult *C. hians* that caused nodules in the muscular layer. F) Adult *Cathaemasia hians*, HE.

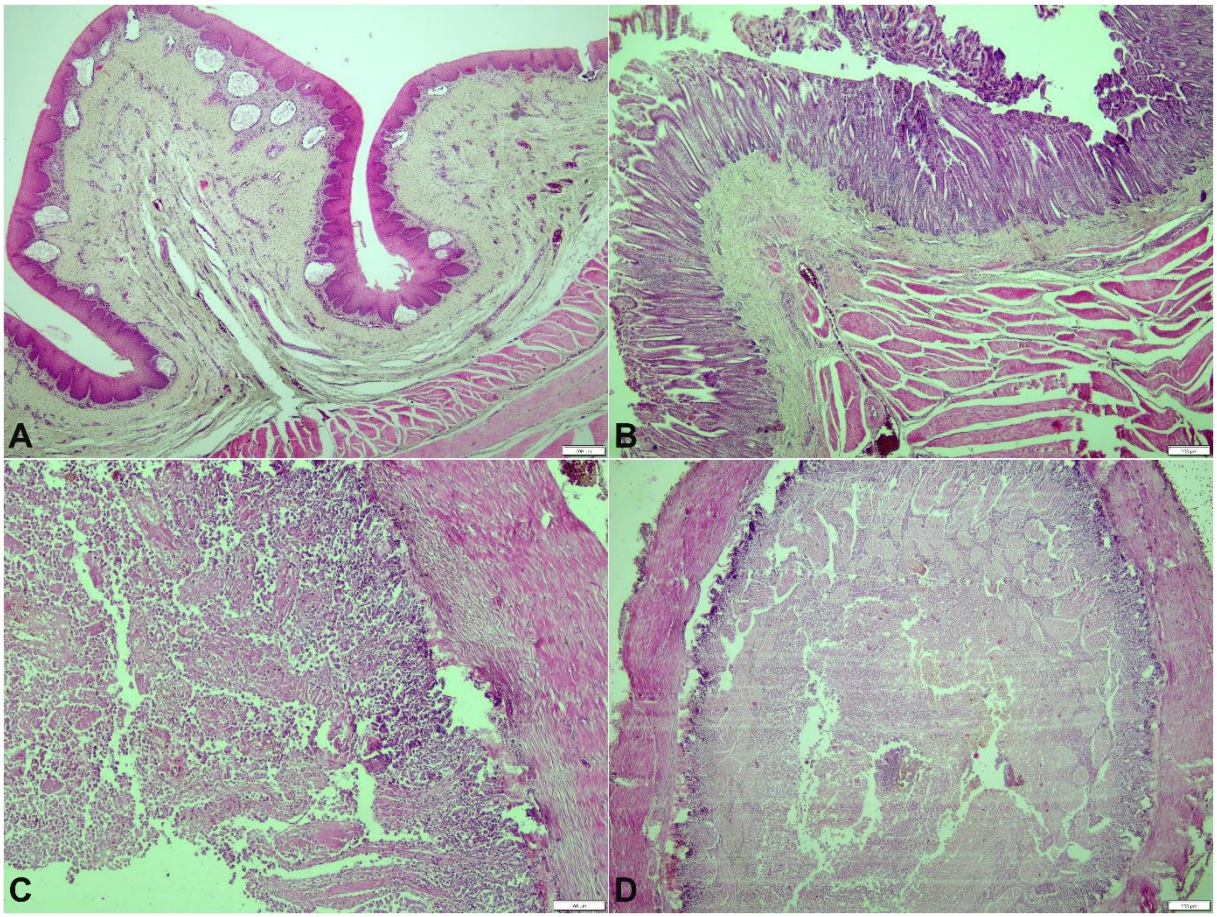

Figure 3. Microscobic findings of white storks. A) No trematode in the oesophagus. B) Degeneration and desquamation in the epithelial layer of the proventriculus. C-D) Only, degeneration, necrosis, and desquamation in the intestine and no parasites. HE.

DISCUSSION

This study focuses on adult Cathaemasia hians infection in a specific bird host, the black stork, and the histopathological changes in the infected bird's intestinal tissues. To the best of our knowledge, there are few studies on both stork and digestive system histopathology. There is a study of histopathological changes in the upper gastrointestinal system in Slovakia (Königova et al., 2015) and a study of histopathological changes in the intestine of white storks in Poland (Michalczyk et al., 2020). Moreover, infection with *C. hians* has been described in injured adult black storks and later in juvenile nesting storks (*Ciconia nigra*) in Spain (Merino et al., 2001). *C. hians* was found in a black stork after colliding with electric cables in southern Portugal (Ramilo et al., 2021). Eight species of helminths, Stephanoprora (Monilifer) spinulosa, Dictymetra discoidea, Schistocephalus solidus, Echinoparyphium sp., Chaunocephalus ferox, Tylodelphys clavata, T. excavata, and Syncuaria ciconiae, have been detected in white storks in Türkiye (Girisgin et al.,2017). The species identification of internal parasites seen in black stork in Türkiye was made for the first time by Işık et al. (Işık et al., 2017). However, to the best of our knowledge, this is the first study conducted on the histopathological findings of parasites in the intestine of storks in Türkiye.

White storks primarily feed in permanent pastures (Carrascal et al., 1993), with arthropods and earthworms (Alonso et al., 1991), furthermore, they now feed in garbage dumps near cities. On the other hand, the endangered black stork has maintained its feeding habits based on amphibia and fish, which are the most common intermediate hosts of *C. hians* (Merino et al., 2001). As the researchers stated, the

difference in trematodes obsrved in white and black storks may be related to their dietary choices. This would explain the severe nodules caused by *C. hians* in the intestine of the black stork. In addition, dietary differences may explain differences in prevalence of bird species.

Cathaemasia hians, became a Cathaemasiidae trematode, a well-known species that has members of the Ciconiidae family as definitive hosts and is commonly found in the oral cavity and occasionally in the oesophagus of these birds (Merino et al., 2001; Liptovszky et al., 2012; Ramilo et al., 2021). In the present study, macroscopically, *C. hians* was not observed in the digestive systems of white storks, whereas this trematode was found only in the oesophagus of black storks. Contrary to what the researchers reported, it was not observed in the oral cavity. Despite the presence of C. hians in the oesophagus, no change in the oesophageal tissue was observed histopathologically. It was considered that this trematode's main affinity is for the intestines. *C. hians* can cause irreversible changes in the digestive tracts of definitive hosts. Massive infections by this parasite can result in serious health problems when combined with cachexia or lowered host immunity (Königova et al., 2015). *C. hians* infections of storks have also been reported to be lethal (Sitko and Heneberg, 2015). Some researchers have reported that nodules in the intestinal walls of post-mortem storks were detected, indicating the presence of *C. hians* (Merino et al., 2001; Michalczyk et al., 2020). Furthermore, Michalczyk et al. (Michalczyk et al., 2020) have indicated that no other pathological changes or internal parasites were found except for nodular enteritis. In this study, the storks were brought in dead and without injury. While the cause of death in black storks was thought to be parasites, the cause of death in white storks had not been found. Similar to the researcher’s findings, there were numerous nodules in the serosa of the intestines and histopathologically visible ulcers caused by parasites in the intestines. In addition, trematodes were observed in the muscular layer of the intestines. Similarly, no other pathological changes were found in the intestine, except for nodular enteritis.

Dicheilonema ciconiae (Nematoda, Diplotriaenoidea) is a helminth found in birds' respiratory systems that parasitizes fish-eating birds (Syrota et al., 2016). *D. ciconiae* can be divided into two groups according to its localization type. The first group comprises parasites of organs related to the external environment that parasitize the eyes, nasal cavities, and air sacs. The second group consists of nematodes that parasitize organs that are not associated with the external environment, such as the abdominal and thoracic cavities, subcutaneous cellular tissue, muscles, joint sac, blood, and lymphatic systems (Saparov et al., 2013). *D. ciconiae* was observed in the thorax and abdominal cavity particularly in the air sacs. It is thought that it passes through the air sacs into the thorax and abdominal cavity. As stated by the researchers, it belongs to the first group and has been regarded as a parasite of organs associated with the external environment.

CONCLUSION

In this study, it was the first time that histopathological changes in the digestive system of storks in Türkiye were defined. Despite the modest number of instances in this study, it was believed that it would be more suitable to report this study, which studies alterations in the digestive systems, as a research paper because stork deaths were uncommon. The presence of trematode eggs and adults in the lumen and tissues of the stork's intestine was associated with a severe nodular inflammatory response. In the case of severe infection by parasites, tissue damage and inflammation lead to malnutrition and reduced body weight. Even severe infections can leave the host vulnerable and cause death. However, it remained unclear which parasite was the cause of death in this study, particularly in the afflicted black stork. Other infectious or poisonous factors were assumed to be the causes of death in white storks.

Acknowledgements
We wish to thank Nermin Işık et al. for providing the optical stereoscope picture of the parasite. Research permission was obtained from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (No: 11402716).

Ethical Approval
This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information, and documents presented in this study were obtained within the framework of academic and ethical rules.

Conflict of Interest
None. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Özgür Kanat.

REFERENCES

Alonso J.C., Alonso J.A., Carrascal L.M. (1991). Habitat selection by foraging white storks, *Ciconia ciconia*, during the breeding season. *Canadian Journal of Zoology*, 69(7): 1957-1962.

Carrascal L.M., Bautista L.M., Lazaro E. (1993). Geographical variation in the density of the white stork *Ciconia ciconia* in Spain: influence of habitat structure and climate. *Biological Conservation*, 65(1): 83-87.

Carrera-Jativa P.D., Morgan E.R., Barrows M., Jimenez-Uzcategui G., Tituana J.R.A. (2020). Free-ranging avifauna as a source of generalist parasites for captive birds in zoological settings: An overview of parasite records and potential for cross-transmission. *Journal of Advanced Veterinary and Animal Research,* 7(3): 482-500. http://doi.org/10.5455/javar.2020.g445

Chaudhary B., &Pandey D.P. (2020). Snapshot view of intestinal parasites in wild birds of Chitwan District, the Southcentral Lowlands of Nepal. *Research Journal of Parasitology*, 15: 1-8. https://doi.org/10.3923/jp.2020.1.8

Colcimen N., Arihan O., Gumusok S., Kilic C.S. (2020). Effect of The Opopanax Hispidus Plant's Aerial Parts Extract on Mice Ovary. *Selcuk Medical Journal*, 36(1): 44-47.

Dörtbudak M.Y., Sağlam Y.S., Dörtbudak M.B. (2019). Sarıprenses balığında (Labidochromis caeruleus) Yoğun parazit enfestasyonuna bağlı bağırsak hasarının histopatolojik incelenmesi. *Harran Üniversitesi Veteriner Fakültesi Dergisi*, *8*(1): 52-56.

Girisgin A.O., Birlik S., Senlik B., Yildirimhan H.S. (2017). Intestinal helminths of the white stork (Ciconia ciconia Linnaeus 1758) from an inter-route site in Turkey. *Acta Veterinaria Hungarica*, 65(2): 221-233. https://doi.org/10.1556/004.2017.022

Işık N., Kanat Ö., Ceylan O., Zerek A., Güçlü F. (2017). Bir kara leylekte (Ciconia nigra) rastlanan iki helmint türü: Cathaemasia hians (Trematod, Cathaemasiidae) ve Dicheilonema ciconiae (Nematod, Diplotriaenoidea). 20. Ulusal Parazitoloji Kongresi (Uluslararası Katılımlı), 25-29 Eylül 2017, Eskişehir, 613.

Karaska D. (2002). Bocian cierny (Ciconia nigra). In: Danko S., Darolova A., Kristin A. (editors) Rozsirenie vtakov na Slovensku, Bratislava, Slovakia, 688.

Königova A., Hrckova G., Molnar L., Major P., Varady M. (2015). *Cathaemasia hians* infection in Black stork in Slovakia: morphological and histopathological study. *Helminthologia*, 52(4): 316-322.

https://doi.org/10.1515/helmin-2015-0050

Liptovszky M., Majoros G., Perge E. (2012). Cathaemasia hians in a Black Stork (Ciconia nigra) in Hungary. *Journal of Wildlife Diseases*, 48(3): 809–811.

Luna L.G. (1968). Routine staining procedures. In: Manual of histologic staining methods of the Armed Forces Institute of Pathology, McGraw-Hill Book Co NY, USA, pp. 33-46.

Merino S., Martinez J., Lanzarot P., Cano L.S., Fernandez-Garcia M., Rodriguez-Caabeiro F. (2001). Cathaemasia hians (Trematoda: Cathaemasiidae) infecting black stork nestlings (Ciconia nigra) from central Spain. *Avian Pathology*, 30(5): 559-561. https://doi.org/10.1080/03079450120078761

Michalczyk M., Sokol R., Gesek M., Mączynski M., Bedzlowicz D. (2020). Internal parasites and associated histopathological changes in deceased white storks from Poland. *Belgian Journal of Zoology*, 150: 71-80. https://doi.org/10.26496/bjz.2020.74

Moreno Manas E., Gonzalvez Juan M., Ruiz De Ybanez Carnero M.D.R., *et al*. (2019). Survey of husbandry practices for bovidae in zoos: the importance of parasite management for reintroduction programmes. *Veterinary Record*, 184(9): 282. https://doi.org/10.1136/vr.104985

Muller M.G. (2009). Parasitic diseases. In: Practical handbook of falcon husbandry and medicine, Nova Science Publishers, New York, pp. 191-222.

Nalubamba K.S., Bwalya E.C., Mudenda N.B., Munangandu H.M., Munyeme M., Squarre D. (2015). Prevalence and burden of gastrointestinal helminths in wild and domestic guineafowls (Numida meleagris) in the Southern Province of Zambia. *Asian Pacific Journal of Tropical Biomedicine*, 5(8): 663-670. http://dx.doi.org/10.1016/j.apjtb.2015.04.009

Nasiri V., Jameie F., Paykari H., Mazhari N., Soltani S., Pashaei M. (2019). Survey of gastrointestinal parasitic infection in Captive Wild Animals of a Central Zoological Garden in Iran. *Journal of Veterinary Science and Research*, 1: 78-91. https://doi.org/10.36811/jvsr.2019.110009

Otegbade A.&Morenikeji O. (2014). Gastrointestinal parasites of birds in zoological gardens in South-West Nigeria. *Tropical Biomedicine*, 31(1): 54-62.

Penczykowski R.M., Laine A.L., Koskella B. (2016). Understanding the ecology and evolution of host-parasite interactions across scales. *Evolutionary Applications*, 9(1): 37–52. https://doi.org/10.1111/eva.12294

Ramilo D.W., Caetano I., Brazio E., *et al*. (2021). Presence of one ecto- and two endoparasite species of the black stork (Ciconia nigra) in Portugal. *BMC Veterinary Research*, 17: 21. https://doi.org/10.1186/s12917-020-02724-6

Saparov K., Akramova F., Azimov D., Golovanov V., Kuchboev A. (2013). Biodiversity of filariae (Nematoda: Filariata), parasites of birds in Uzbekistan. *Turkish Journal Zoology*, *37*(6): 746-752. https://doi.org/10.3906/zoo-1106-3

Sitko J.&Heneberg P. (2015). Composition, structure and pattern of helminth assemblages associated with central European storks (Ciconiidae). *Parasitology International*, 64: 130-134. http://dx.doi.org/10.1016/j.parint.2014.11.004

Soltysiak Z., Rokicki J., Kantyka M. (2014). Histopathological diagnosis in parasitic diseases. *Annals of Parasitology*, 60(2): 127–131.

Syrota Y.Y., Kuzmin Y.I., Lyaskivskiy V.N., Kobylinsky V.V., Vasylkivska I.B. (2016). First Record of Dicheilonema Ciconiae (Nematoda, Diplotriaenoidea) From Ciconia Nigra (Aves, Ciconiidae) in Ukraine. *Vestnik zoologii*, 50(4): 379-382. https://doi.org/10.1515/vzoo-2016-0044

Tylkowska A., Stapf-Skiba A., Rzad I., Jezewski W. (2019). Helminths of white stork Ciconia ciconia (L., 1758) in north-eastern Poland. *Annals of Parasitology*, 65, Supplement 1, 45-46.