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THE FIRST STUDY OF THE ENDOPARASITIC FAUNA OF MUTE SWANS (*CYGNUS OLOR*) IN THE NORTHERN PART OF SERBIA

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Abstract

In the Northern part of Serbia, which is part of Pannonian Basin, mute swan (*Cygnus olor*) population has notably increased over the last few decades. Like other birds from *Anatidae* family, mute swans are a host of numerous endoparasite species. The aim of this study was to acquire the data on identification and prevalence of endoparasites in mute swans in the Republic of Serbia, as that information is lacking. Individual faecal samples of sixty-eight adult mute swans were examined for the presence of endoparasites. Coprological examination was performed using flotation and sedimentation technique with saturated ZnSO₄ solution. The samples were collected from December 2016 to March 2017, during epizootic of highly pathogenic avian influenza H5N8 in the Republic of Serbia. Dead mute swans were collected from twelve different locations in the Northern part of Serbia. Altogether, 39.7% of the examined fecal samples contained different parasites. The endoparasitic fauna was diverse and included 3 species of nematodes, 2 cestodes species, one trematode and one protozoan species. Nematodes were the most prevalent helminthes and among these the most frequent nematode species found was *Heterakis dispar* (17.6%), followed by *Echinuria uncinata* (5.8%) and *Ascaridia* spp. (4.4%). Four mixed infections were found, where double infection was the most prevalent. No endoparasites were found in faecal samples of mute swans collected from the locations Sombor and Titel. As this study covers a small population of mute swans, more detailed studies need to be carried out on a larger population

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in order to gain an insight into the diversity and prevalence of endoparasites in the Republic of Serbia.

Key words: coprological examination, endoparasites, mute swans, nematodes

PRVO ISTRAŽIVANJE FAUNE ENDOPARAZITA KOD LABUDOVA GRBACA (*CYGNUS OLOR*) NA PODRUČJU SEVERNOG DELA SRBIJE

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Kratak sadržaj

U severnom delu Republike Srbije koji pripada Panonskom basenu, populacija labudova grbaca (*Cygnus olor*) značajno je porasla tokom poslednjih decenija. Kao i većina drugih ptica iz porodice *Anatidae*, labudovi grbci su domaćini različitih endoparazitskih vrsta. U literaturi ne postoje podaci o endoparazitima labudova u Republici Srbiji, stoga je cilj ovog istraživanja bio identifikacija i prevalenca endoparazita kod ove vrste. Parazitološki je ispitano šezdeset osam pojedinačnih uzoraka fecesa poreklom od odraslih labudova grbaca. Koprološki pregled je izvršen metodom flotacije i sedimentacije zasićenim rastvorom ZnSO₄. Uzorci su prikupljeni tokom epizootije visoko patogene avijarne influence H5N8 u Republici Srbiji, u periodu od decembra 2016. do marta 2017. godine. Uginuli labudovi prikupljeni su sa dvanaest različitih lokacija u severnom delu Srbije. Različite parazitske vrste detektovane su u 39.7% pregledanih uzoraka fecesa. Identifikovane su tri vrste nematoda, dve vrste cestoda, jedna vrsta trematoda i jedna vrsta protozoa. Nematode su utvrđene kao helminti sa najvećom prevalencom, a najučestalije detektovane vrste bile su *Heterakis dispar* (17.6%), zatim *Echinuria uncinata* (5.8%) i *Ascaridia* spp. (4.4%). Utvrđene su četiri mešovite infekcije, gde je dvostruka infekcija bila najčešći nalaz. Paraziti nisu detektovani iz uzoraka fecesa poreklom od labudova sa lokacija Sombor i Titel. Uzimajući u obzir činjenicu da je u ovom

istraživanju obuhvaćena mala populacija labudova grbaca u Republici Srbiji, potrebno je sprovesti detaljnije studije na većoj populaciji, kako bi se dobio sveobuhvatniji uvid u raznolikost i rasprostranjenost endoparazita.

Ključne reči: koprološki pregled, endoparaziti, labudovi grbci, nematode

INTRODUCTION

It is known than mute swan (*Cygnus olor*, Gmelin, 1789) population started to colonize wetlands in Northern parts of Serbia in the early 1980s (Hulo, 1997). Today there is no precise data about the recent size of the mute swan population, but it is known that in the last three decades they mostly overwinter in Bačka Podunavlje, Northern part of Serbia. Also, there is no detailed survey on all breeding sites or the number of breeding pairs in whole country. However, it is known that the mute swan population in the Republic of Serbia has increased during the last three decades (Tucakov, 2005).

Mute swans are large herbivorous birds, belonging to Anseriformes order, Anatidae family. The Anseriformes order is primarily associated with water and wetland habitats, while the Anatidae are typical waterfowl. Waterfowl characteristic to aggregate in large numbers during breeding season or winter migration can lead to the transfer of disease-causing organisms, including different parasites. Parasitic diseases of waterfowl are common, but they do not cause high mortality rate. However, parasites can contribute to increased mortality in cases of other disease outbreaks (Olsen, 2009).

It is shown that diet and feeding habits play a key role to the parasite fauna in birds. Also, habitat can play an important role in creating parasite assemblages in birds. Some host species that have wider geographic distribution tend to have more diverse parasite community structure, including waterfowl and migratory birds, which distinguish them from non-migratory bird species (Koprivnikar and Leung, 2015). However, it is still not clear which factors most significantly affect the diversity and composition of parasite assemblages in migratory birds and whether this distinguishes them from non-migratory birds (Leung and Koprivnikar, 2016). In Europe, the three different swan species can be found: Mute Swan (*Cygnus olor*), Whooper Swan (*Cygnus cygnus*) and Bewick's Swan (*Cygnus columbianus bewickii*). Out of the three European swan species, mute swans are the most sedentary, and their movements rarely

exceed tens of kilometres (Scott and Rose, 1996; Waldenström et al., 2017).

To our knowledge, there are no literature data about the biodiversity of parasites in mute swans in the Republic of Serbia. Considering its partially migratory behaviour and their strong association with aquatic habitats, we found it very important to establish the extensity and prevalence of endoparasitic infections in mute swan populations in Northern part of Serbia.

MATERIAL AND METHODS

Uncommon high morbidity and mortality of mute swans were observed in Northern part of Serbia between December 2016 and March 2017. During that period, dead mute swans were collected from twelve different locations (Figure 1) and submitted to the Scientific Veterinary Institute „Novi Sad“, in order to determine the cause of death. Most of dead mute swans were found along the shores of Danube armlet. These areas are characterized by shallows and inlets of the Danube River, which are a temporary home for thousands of migratory and resident aquatic birds. All swans were examined by a full necropsy according to a standard protocol and gross lesions were recorded. Due to the emergence of highly pathogenic avian influenza H5N8 outbreaks in the autumn of 2016 in most European countries, as well as a large percentage of mortality in wild aquatic birds, there was a suspicion of this disease. Different tissue samples (lungs, heart, kidney, intestine, spleen, pancreas and brain) were collected from each bird for detection of avian influenza virus and other differential laboratory investigations including histopathology and parasitology. The gastro-intestinal tracts of all 68 mute swans were removed and cut into parts. The intestines and gizzards were further carefully slit open and examined in detail. Faecal samples from distal part of the intestine of each bird were collected, labelled, placed in clean plastic containers and stored until use at 4°C. Coprological examination was performed in parasitology laboratory at Institute of Veterinary Medicine of Serbia, Belgrade using flotation and sedimentation technique with saturated ZnSO₄ solution. Adult parasites were examined using light microscopy after being clarified in lactophenol. The identification of the parasites was based on morphological criteria and was carried out following the keys of Anderson (2000), Cole and Friend (1999), Taylor et al., (2007) and Rysavy and Cerna (1988).



Figure 1. Geographical distribution of tested mute swans in the Northern part of Serbia.

RESULTS

All dead mute swans were found to be positive for the highly pathogenic avian influenza strain (HPAI) H5N8. Generally, all swans were in good body condition during necropsy, with thick subcutaneous and cavitary fat, with mild or no external lesions, which is considered normal in the winter season. Gross pathology included hyperemia, hemorrhages, necrosis in most of visceral organs and the lesions were characteristic for HPAI infection (Božić et al., 2016; Božić et al., 2018).

Out of 68 mute swans examined, infection with endoparasites occurred in 39.7% of mute swans (27/68). The eggs of the following helminths classes were detected: Cestodes, Nematodes, Trematodes and one protozoan species. Four mixed infections were established, where double infection was the most

prevalent and detected in 19.1% of the examined swans. Among the observed helminthes, the predominant parasites were nematodes. Out of the three identified nematode, *Heterakis dispar* was the most prevalent, found in 12 birds (17.6%), followed by *Echinuria uncinata* in 4 birds (5.8%) and *Ascaridia* spp. in 3 birds (4.4%). Two cestoda were identified: *Dilepis undula* in one mute swan (1.4%), and *Cladogynia bulbocirrosus* in 3 birds (4.4%). One trematode species was found, *Apatemon gracilis* in one swan (1.4%). Finally, only one protozoan parasite was found – renal coccidia *Eimeria christianseni* in 3 examined mute swans (4.4%). No intestinal parasites were found in faecal samples of mute swans collected from Sombor and Titel. The parasites found in mute swans and their prevalence is presented in the Table 1.

Table 1. Extensivity and prevalence of endoparasites of tested mute swans in the Northern part of Serbia.

Location	Number of birds examined	Parasite						
		Cestodes		Nematodes			Trematodes	Protozoa
		<i>Dilepis undula</i>	<i>Cladogynia bulbocirrosus</i>	<i>Echinuria uncinata</i>	<i>Heterakis dispar</i>	<i>Ascaridia</i> spp.	<i>Apatemon gracilis</i>	<i>Eimeria christianseni</i>
Zasavica	1	1/1	-	-	-	-	-	-
Titel	1	-	-	-	-	-	-	-
Kanal DTD	17	-	-	1/17	2/17	-	-	-
Žabalj	8	-	-	-	-	1/6	-	-
Futog	3	-	2/3	-	2/3	-	-	-
Palić	10	-	-	-	4/10	-	-	-
Bač	3	-	1/3	-	-	-	1/3	-
Kovilj	10	-	-	2/10	3/10	1/10	-	-
Srbobran	1	-	-	-	-	1/1	-	-
Višnjićevo	3	-	-	-	1/3	-	-	-
Sombor	2	-	-	-	-	-	-	-
Beograd	7	-	-	1/7	-	-	-	3/7
Total	68	1	3	4	12	3	1	3

(-) – not detected

DISCUSSION

Free-ranging wild birds, primarily migratory birds, are capable of transmitting parasitic diseases to greater geographical distances, due to their interference with other non-migratory birds. Mute swans in this study were collected from wintering areas for migratory birds, so the cohabitation with these birds presumably contributes to the composition and structure of the parasitic fauna. Research on parasitic fauna of mute swans is mostly conducted in Europe, New Zealand, and Canada (Papazahariadou et al., 1994; Papazahariadou et al., 2008; Pennycott, 1998; Manno et al., 2016; Seegar 1979; Jennings et al., 1961; Sanford, 1978). There are some studies in the Republic of Serbia regarding parasitological examinations in zoo birds of the Anatidae family (Ilić et al., 2018), but to our knowledge, there is no literature data regarding parasitic fauna of mute swans. This report is the first description of endoparasitic fauna in mute swans located in the Northern part of Serbia.

A total of 39.7% of mute swans in this study were positive for parasite infection and nematodes were the most prevalent helminthes. Detected parasitic helminths were from Cestodes, Nematodes and Trematodes classes, and only one protozoan species was diagnosed - renal coccidia *Eimeria christianseni*. Few birds were harboring multiple nematode species and among them a species from Heterakidae family, *Heterakis dispar*, was the most commonly found (17.6%). The same nematode species was detected in small intestine of black necked swans from southern America (Gonzalez-Acuna et al., 2010). Adult worms of the *Heterakis* genus generally live in the lumen of the ceca of birds. Three species are known to be prominent in poultry: *H. gallinarum*, *H. dispar*, and *H. isolonche* (Park and Shin, 2010), but some species from *Heterakis* spp. genus such as Dalmatian Pelican, Grey Heron, Sea Gulls and Little Owl are also found in wild birds (Papazahariadou et al., 2008). According to the data from Serbian zoos, heterakiosis was diagnosed in 12.74% and 2.56% of the captive birds examined mainly as mixed infection with coccidiosis, capillariosis, askariidiosis and trichostrongylidosis (Ilić et al., 2018).

The nematode *Echinuria uncinata* was detected in 5.8% of mute swans. This nematode was reported earlier in mute swans originating from Northern Greece (Papazahariadou et al. 1994) as well as in another Greek study (Papazahariadou et al., 2008). When *E. uncinata* was first recorded in New Zealand (Clark, 1979), its pathogenicity was indicated and it is regarded as the most devastating parasite of waterfowl in Russia. In general, this species is considered to be highly pathogenic to anatid birds (Silveira et al., 2006). However, in the present work, such condition was not observed and overall prevalence was low.

In the current study, other detected nematode included undefined species from genera *Ascaridia* spp. in 4.4% of mute swans. Ascarids do not normally cause severe pathogenic effects. However, they can cause clinical disease and even death in a case of high intensity of infection (Papini et al., 2012). Ascariidiosis were diagnosed with the overall prevalence of 10.78% and 10.25% in captive birds from Serbian zoos (Ilić et al., 2018), as well as 6.8% of examined birds from Italian zoo (Papini et al., 2012).

Literature data confirms findings of many other nematodes in mute swans and other wild swans that were not diagnosed in our research, comprising species from *Amidostomum* genera (*Amidostomum anseris*), *Capillaria* (*Baruscapiharia obsignata*, *Eucoleus dispar*) (Papazahariadou et al., 2008; Tamaru et al., 2015; MacNeill, 1970), *Acuaria* (*Acuaria uncinata*), and *Tetrameres* (*Tetrameres fissispina*) (Jennings et al., 1961).

In the current study, two cestoda species were identified: *Dilepis undula* in one mute swan and *Cladogynia bulbocirrosus* in 3 examined swans (4.4%). Typically, *Dilepis undula* is a parasite of passeriform birds. Some authors recorded it in blackbirds (*Turdus merula*, *Turdus viscivorus*), hooded crows (*Corvus cornix*), Euro-Asian sparrows (*Passer montanus*) and starlings (*Sturnus vulgaris*) in southern Bulgaria (Marinova et al., 2013). It was also identified in blackbirds in Poland (Rzad et al., 2014).

The cestode *Cladogynia bulbocirrosus* was detected in 3 examined swans (4.4%). To our knowledge, the only literature data on the prevalence of this cestode species in swans was reported in three black-necked swans (*Cygnus melancoryphus*) in Vienna Zoological Gardens (Gonzalez-Acuna et al., 2010). According to the data from Egypt, species from *Cladogynia* genus - *Cladogynia phoeniconiadis* were reported in ducks in low prevalence (2.7%) (Aboulaila et al., 2011). Also, cestodes of the *Cladogynia* genus were found incidentally in free-ranging flamingos in Mexico (Aguirre et al. 1991) and low prevalence was recorded in birds in *Phoenicopteridae* family (Papazahariadou et al., 2008).

In mute swans from this study, only one trematode species was detected - *Apatemon gracilis* in one swan. *Apatemon gracilis* is an intestinal trematode that was frequently reported in ducks in certain geographic areas of Europe and it was also found in the intestine of various wild birds. Ducks are its main hosts (Liu et al., 2018). The life cycle of this fluke requires two intermediate hosts: the first is a freshwater snail and the second includes frogs and freshwater fish in addition to snails. A final host is infected by feeding on the second intermediate host containing metacercariae. The low prevalence of this fluke in our study may be related to cold winter, bad weather conditions and probably absence of intermediate hosts. Reports of trematode-induced mortalities of

swans are scarce. Some authors described lethal ulcerative hemorrhagic enteritis in mute swans caused by some trematode species such as *Echinoparyphium recurvatum* and *Sphaeridiotrema globules* (Roscoe and Huffman, 1982).

As for protozoan species, one species was diagnosed - renal coccidia *Eimeria christianseni*. Low prevalence of renal coccidia could probably be explained due to a small number of mute swan kidneys examined (10.3% of the tested swans). Renal coccidiosis is caused by protozoal parasites that infect the kidneys and associated tissues. Most of the coccidia that infect the tissues in most bird species belong to *Eimeria* spp. As with most other parasitic infections, this infection is not synonymous with clinical or apparent disease. Asymptomatic infections are far more common than those that are severe and cause mortality (Cole and Friend, 1999). However, in some cases these parasites can cause serious health problems (Giacomo et al. 1997; Pennycott et al. 1998).

CONCLUSION

The present survey on endoparasitoses is a valuable initial research work which gives an insight into the endoparasitic fauna of mute swans in the Republic of Serbia. Even though the assessment included statistically a small number of animals, it showed that the endoparasitic infections are present in the Northern part of Serbia, and that they are diverse and include nematodes, cestodes, trematodes and protozoa. The results will surely be valuable for preparing further research plans in this field.

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Author's contributions:

BĐ drafted the manuscript, carried out literature research, performed necropsy and sample collection; IP carried out the parasitological examination; MP participated in the design of the study; MS, MP and JP did the reviewing, editing and supervision; VP revised manuscript critically and gave the final approval of the version to be published.

Competing Interests

Authors declared no conflict of interests regarding the present paper.

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