

PATHOLOGICAL AND PARASITOLOGICAL FINDINGS IN SERBIAN GREAT CORMORANTS INFECTED BY *CONTRACOECEUM RUDOLPHII* SENSU LATO

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The carcasses of ten great cormorants (*Phalacrocorax carbo*) found in one colony in the province of Vojvodina were necropsied and examined for parasites. The study aimed to identify helminths and histopathologically evaluate the associated tissue damage. Numerous nematodes were observed, either free in the the lumen of the ventriculus or deeply embedded in the ventricular mucosa of all the cormorants examined. The collected parasite samples were morphologically identified as *Contraecaecum rudolphii* sensu lato. The main pathological findings included a thickening of the ventricular wall, while histopathology revealed a mild inflammatory infiltrate in the adjacent tissue and a connective tissue capsule around the parasite. Given that the parasites in this bird species still need to be thoroughly studied in our country, particularly the pathomorphological changes they cause, this study represents the first attempt to obtain a comprehensive understanding picture of the nematode infection and the pathological changes resulting from the presence of this parasite.

Keywords: *Contraecaecum rudolphii*, great cormorant, pathomorphological changes, Serbia

INTRODUCTION

Wild migratory birds directly contribute to the spread of pathogenic agents, and migration undoubtedly increases their exposure to new pathogens. Migratory birds can get pathogens directly, during interaction with other infected birds, most often at feeding grounds and stopover areas [1]. During these migrations, birds have the potential to spread a wide variety of pathogens to distant regions, many of which are of public health importance [2].

Great cormorants (*Phalacrocorax carbo*) play a significant role in the spread of pathogens, as they have been proven to carry, transmit and distribute specific pathogens,

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including parasites [3,4]. The great cormorant is the most widespread species of the genus *Phalacrocorax* inhabiting all continents except South America and Antarctica. It belongs to migrating birds, and the European population of this species has significantly increased in the last three decades [3]. In Serbia, the breeding population of great cormorants is also increasing. According to the most recent official census from 2012, 20 colonies were recorded comprising approximately 2000 pairs [5]. Under the Rulebook on the Proclamation and Protection of strictly protected and protected wild species of plants, animals and mushrooms [6], the great cormorant is classified as a protected species in our country.

Since great cormorants are social birds, they nest in colonies on land and gather in flocks, with a relatively large feeding area. Great cormorants are swimmers and divers that feed exclusively on various types of fish. They play a significant sanitary role in regulating fish populations removing parasite-infected fish from aquatic ecosystems [7]. On the other hand, they also contribute to the spread of parasites in aquatic ecosystems. The large amounts of feces produced by cormorants contain developmental stages of parasites which continue their developmental cycle in the water [8]. Certain types of parasites associated with cormorants can potentially be pathogenic to humans, domestic animals, and commercial fish species [9].

Although endoparasites, particularly nematodes, are common in wild birds, more research is needed to understand the changes they cause in this species. In the case of great cormorants, only one study has examined the prevalence of gastrointestinal nematodes in free-ranging cormorants from Serbia [10]. However, no study has been conducted regarding the pathohistological findings of parasite-related changes in bird organs or the morphological characteristics of the parasites. Therefore, it is crucial to describe the parasites' characteristics and the pathohistological changes they cause, which result from a normal immune response or from damage to the host's homeostatic mechanisms [11]. Thus, the present study aimed to survey parasitic nematodes in the stomach of great cormorants (*Phalacrocorax carbo*) collected from a colony in Vojvodina Province. The study also sought to establish and describe the pathohistological changes in the gastric wall caused by these parasites and potential pathological consequences of gastric parasitism.

MATERIAL AND METHODS

Ten carcasses of adult great cormorants were found and collected from January to July 2018 at a fishpond near the village Ruski Krstur (West Bačka District, The Province of Vojvodina, northern Serbia). The dead birds were discovered entangled in fishermen's nets or along the shore during this period. The carcasses were collected and sent to the Scientific Veterinary Institute "Novi Sad" for necropsy and detailed parasitological investigation, where the birds were immediately examined upon delivery. The cormorants' gastrointestinal system was removed, and the esophagus,

proventriculus, ventriculus, and intestines were examined separately. Macrolesions were photographed.

After opening the proventriculus and ventriculus, all nematodes not attached to the ventriculus wall were carefully removed and placed in Petri dishes containing 70% alcohol. Once the nematodes were removed, samples of the ventriculus wall were taken for histopathological analyses. Full-thickness samples of the ventricular wall from infected birds were used for histopathology. The sampled tissues were fixed in 10% buffered formaldehyde for at least 72 hours. After fixation, the tissues were routinely processed and embedded in paraffin. Sections were cut at 5 μ m and stained with hematoxylin and eosin. The recovered parasites were extensively washed in physiological saline, fixed in 70% ethanol and cleared in lactophenol solution overnight. The specimens were examined under the microscope (Olympus CX23 and Olympus BX51). Photographs of certain morphological structures of the parasites were taken by a digital camera DinoEye Dino-Lite (AM7023-R4) and Olympus Color View III. The parasites were differentiated by their stage of development and sex. Adult nematodes were identified at the species level based on the morphological keys and descriptions provided by Baruš *et al.*, Amato *et al.* and Li *et al.* [12-14].

RESULTS AND DISCUSSION

Gross examination of ten ten adult great cormorants revealed generally good body condition, well-developed pectoral musculature, and well to moderately present visceral fat, except for one bird that was slightly macerated. The ventriculi of six birds were dilated and enlarged to approximately 2–3 times their normal size due to thickening of the muscle layer. Necropsy showed that all great cormorants had numerous adult nematodes in the ventriculus, either attached or free in the lumen (prevalence rate was 100%) (Figure 1A). In five cases, fewer than 10 nematodes were present free in the lumen of the proventriculus (Figure 1B).

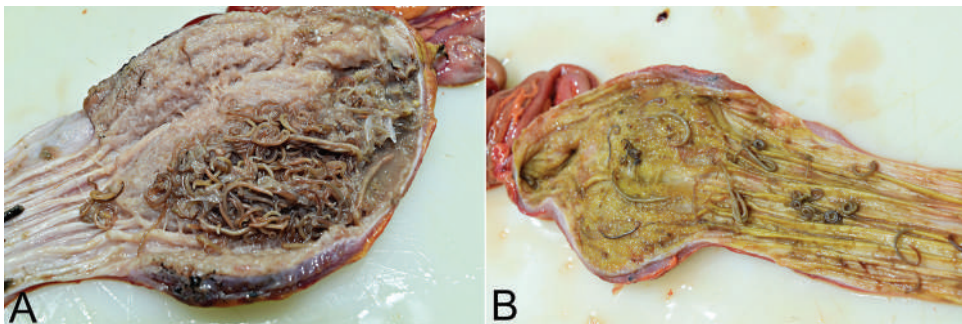


Figure 1. (A) Numerous nematodes *Contracoecum rudolphii* s.l in the lumen of the ventriculus and proventriculus (B).

No parasites were found in the esophagus or intestines. In other cases, the good physical condition of cormorants has also been described in individuals who were infected with this parasite, whether it was free in the proventriculus or attached to its wall [15]. The stomachs of most examined birds contained very little food; in two cases, whole or slightly digested fish was found. The nematodes were associated with the surface of the ventriculus mucosa, with some penetrating the mucosa and extending deep into the submucosa, muscle layer, and serosa (Figure 2A). The ventriculus wall was severely and extensively thickened in birds with a heavy parasite infestation. Irregular red areas of the ventriculus were observed in areas where higher concentrations of parasites were found (Figure 2B). Unlike our findings, where parasites were only found in the proventriculus and ventriculus of cormorants, Cammilleri et al. [16] observed parasites also in the intestines, while Rokicki et al., and Yakovleva et al. [4,15] reported their presence in the esophagus. No notable gross lesions were observed in other organs during necropsies.

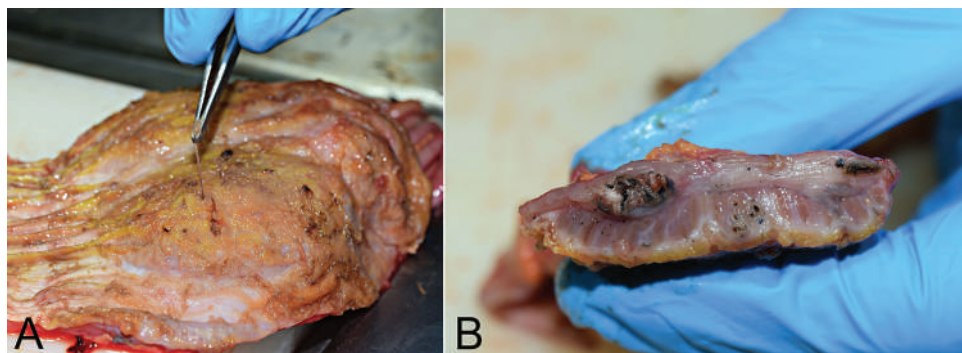


Figure 2. (A) Adult nematode *Contracoecum rudolphii* s.l. deeply attached to the ventricular wall of the great cormorant (B) Cross-section of the ventriculus showing multifocally embedded adult nematode *Contracoecum rudolphii* s.l. in mucosa, submucosa and muscular layer.

During tissue processing, parasites were detached from the muscle layer of the ventriculus in two cases. The koilin layer was disrupted by numerous adult nematodes. Multiple cross and longitudinal sections of adult nematodes as well as parasite larvae stages, were visible in the examined ventriculi sections. Most adult female parasites were sexually mature, containing numerous oval-to-ellipsoid, thick-walled embryonated eggs in the uterus (Figure 3). Histological examination in a few cases revealed a mild inflammatory infiltrate in the adjacent tissue, consisting mainly of eosinophils and lymphocytes, with a few diffusely scattered heterophils also present. Literature data indicate that moderate lymphocytic, heterophilic, and eosinophilic infiltrations are most often present in bird tissues near the attachment sites of various nematodes [17,18]. However, in most cases, inflammation was absent, with only a connective tissue capsule surrounding the parasite. Similar to our findings, Sato et al., and Carvalho et al., [19,20] described the absence of a tissue reaction in the ventriculus

due to the presence of the *Contraecocum sp.* In contrast, other cases have reported that the presence of *Contraecocum sp.* in the ventriculus is accompanied by moderate to severe inflammation, including granuloma formation with nematode remains and necrotic debris at the center, surrounded by epithelioid and foreign-body type giant cells [15,21]. Different histopathological findings and the severity of the lesions can most likely be explained by the time intervals that have passed since the parasite attached to the ventriculi mucosa.

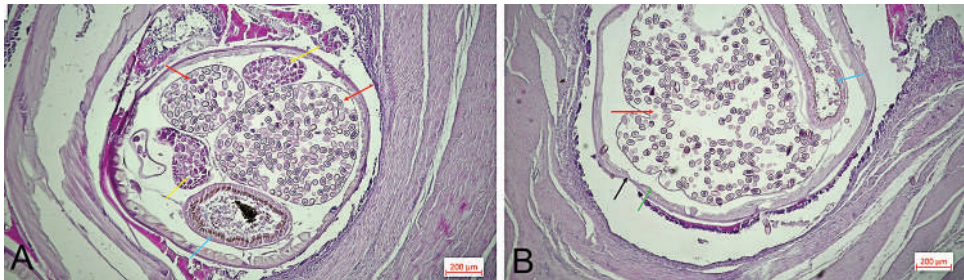


Figure 3. Histological section of the ventriculus of great cormorants parasitized with female nematode *Contraecocum rudolphii* s.l. **(A)** uterus filled with eggs (red arrow), transversal section of the ovary (yellow arrow) and lumen of the intestine (blue arrow pointing at intestinal wall); **(B)** uterus filled with eggs (red arrow), intestinal wall (blue arrow), cuticle (black arrow) and muscle layer of the body wall (green arrow).

A total of 135 nematode *Contraecocum rudolphii* s.l. specimens were detected at necropsy, comprising 71 females, 61 males and three larvae (Figure 4).

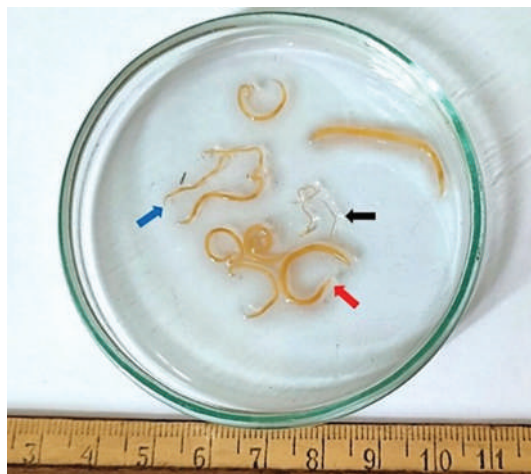


Figure 4. Nematodes *Contraecocum rudolphii* s.l. in a Petri dish – females (red arrow), males (blue arrow) and larvae (black arrow).

Adult specimens were yellowish to brownish in color. The anterior end had three prominent lips surrounding the mouth opening, each slightly wider than long (Figure 5 A, B). The anterior part of the lips formed two lobes, each divided into lateral and medial lobes. The interlabium was well-developed and bifurcated at the distal end. A cephalic collar with numerous annulations posterior to the lips was present (Figure 5 B). The body length of the females ranged from 27.4 to 38.6 mm. The conical posterior end of the body had a rounded tip (Figure 5 C). The uterus was filled with oval eggs measuring approximately 50 x 60 μm (Figure 5 D).

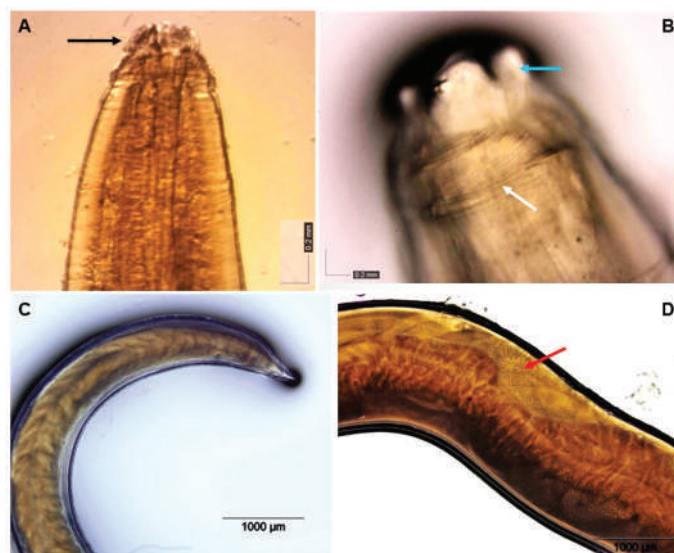


Figure 5. Female *Contracaecum rudolphi* s.l. **(A)** Anterior end with three lips (black arrow); **(B)** Anterior end with three lips (blue arrow) and cephalic collar two rows of precloacal papillae (white arrow); **(C)** Posterior end; **(D)** Body with uterus filled with eggs (red arrow).

The body length of the males ranged from 17.3 to 23.4 mm. The posterior end of the males was blunted and bore two subequal spiculae (Figure 6 A). The cuticle showed fine striations (Figure 6 B). Numerous pairs of precloacal papillae were arranged in two longitudinal rows (Figure 6 B), and seven pairs of postcloacal papillae were present. Additionally, the males had two nearly equal, apically subpointed spicules, approximately 7 mm long, bearing longitudinal alae (Figure 6 C, D).

The results of this study indicate that the intensity and prevalence of *Contracaecum rudolphi* s.l. infestation in the great cormorant colony on the examined fishpond was very high. The prevalence and intensity of infection were similar to those reported for cormorants in Poland by Biedunkiewicz et al. [3]. According to several authors [15,22,23], such high levels of parasitic infestations can lead to disorders in digestion and absorption of feed, and when combined with other stress factors, including bacterial infections or xenobiotics, may contribute to death of the bird [3]. However, the

absence of severe lesions and the good condition of the severely infected cormorants in this study suggest that these infections can be well tolerated by these birds.

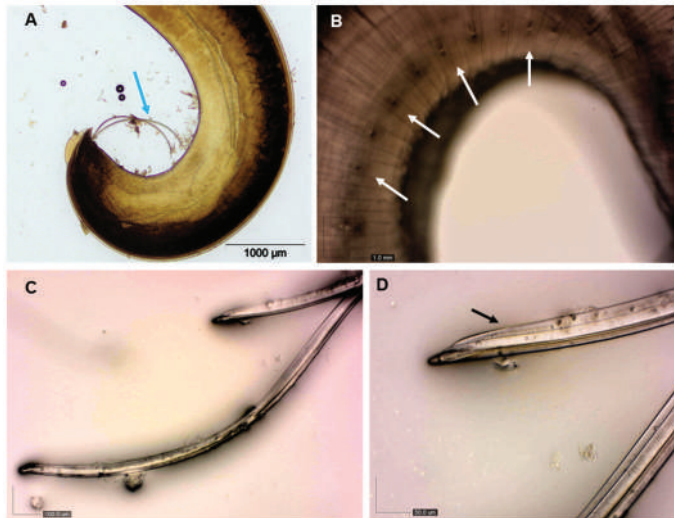


Figure 6. Male *Contracaecum rudolphii* s.l. (A) Blunted posterior end with two subequal spicules (blue arrow); (B) Posterior end with two rows of precloacal papillae (white arrows) and striated cuticle; (C, D) Apically subpointed spicule with with longitudinal allae (black arrow).

The role of cormorants in disseminating parasites, to which they are the final host, has been well documented in the literature. Parasites frequently found in the gastrointestinal tract of cormorants include nematodes of the genus *Contracaecum* (Nematoda: Anisakidae) and the tapeworm *Paradilepis scolecina* [8,13,23-25]. Although preliminary, our findings, provide data on the histological and gross alterations caused by *C. rudolphii* s.l. in great cormorants in Serbia. This report can have a particular clinical and epidemiological significance because more extensive studies regarding the infection of *C. rudolphii* s.l. in great cormorants (*Phalacrocorax carbo*) in Europe have been conducted only in Poland [25,26], Czech Republic [27], Baltic region [28] and Italy [16,29,30,31]. Furthermore, these studies are especially important as *C. rudolphii* infection is now considered an emerging food-borne zoonotic disease [32], with humans at risk of infection through accidental consumption of infected raw or improperly processed fish containing larval stages of *Contracaecum* spp. [33]. To obtain a more comprehensive understanding of the parasite composition and quantitative infection indicators in cormorants and other bird species, research should be expanded to cover a larger area of our country and include samples from more distant sites. Since carcasses of wild birds are often difficult to obtain, every opportunity to collect material should be utilized.

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Authors' contributions

BĐ drafted the manuscript, carried out literature research, performed necropsy, examined and described gross and histopathological lesions, took photography; IV carried out histopathological studies and critically revised the manuscript; DB participated in parasitological study and data analysis; MP and MP performed necropsy, participated in the design of the study; DT helped to draft the manuscript; MĆ has been involved in collecting samples, revised the manuscript and has given the final approval of the version to be published. All authors read and approved the final manuscript.


Declaration of conflicting interests


The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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INFEKCIJA VELIKIH KORMORANA U SRBIJI PARAZITOM *CONTRACOECCUM RUDOLPHII* SENSU LATO – PARAZITOLŠKI I PATOLOŠKI NALAZ

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Uzorci deset leševa velikog kormorana (*Phalacrocorax carbo*) pronađenih u jednoj koloniji u Vojvodini su obdukovani i parazitološki ispitani. Cilj ovog istraživanja bio je da se identifikuju helminti, opišu patohistološke promene i utvrde oštećenja tkiva koje je povezano sa parazitskom infekcijom. Brojne nematode uočene su slobodne u lumenu mišićnog želuca ili su penetrirale u dublje slojeve mukoze mišićnog želuca kod svih ispitanih kormorana. Detektovani paraziti su morfološki identifikovani kao *Contracoecum rudolphii* sensu lato. Glavni makropatološki nalaz bio je zadebljanje zida mišićnog želuca, dok je patohistološkim ispitivanjem promenjenog tkiva želuca uočen blagi inflamatorni infiltrat i vezivno-tkivna kapsula oko parazita. Uzimajući u obzir činjenicu da su paraziti ove vrste ptica nedovoljno i nepotpuno istraženi u našoj zemlji, ovo istraživanje predstavljalo je prvi pokušaj da se dobije potpunija slika o infekciji ovim nematodama i posledičnim patološkim promenama.