Heavy infection with *Contracaecum rudolphii* (Nematoda) in the digestive tract of the great cormorant: Histopathology and new insights on interaction between worm and avian gut microbiota

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We provide the necropsy findings on the anisakid nematode infection of the great cormorant, *Phalacrocorax carbo sinensis*, from the coast of Canneviè lagoon (Northern Adriatic Sea, Italy). Cormorants are very mobile, piscivorous and top-predator birds that consume a wide variety of fish species. Furthermore, they are highly adaptable to changes in marine and riverine prey availability across coastal and inland habitats. Six freshly dead adult great cormorant *P. carbo sinensis* were examined on two occasions during the wintering season 2025. All 6 *P. carbo sinensis* (prevalence 100%) were infected with nematodes of the genus *Contracaecum* with the intensity of infection ranging from 39 to 550 worms per host (212.8 ± 80.7; mean ± S.E.). Co-occurrence of adult and L4 specimens of *Contracaecum* was found in all 6 great cormorants, but the vast majority of worms encountered in each single bird were adult individuals. A subsample of the *Contracaecum* were characterized at species level using a diagnostic key based on the RFLP of two ribosomal regions, the nuclear ITS and the mitochondrial rrnS (12S). Over 80% were *Contracaecum rudolphii* B and about 16% were *C. rudolphii* A. Endoparasitic helminths can induce inflammation and changes in the structure and function of the infected organ. The most parasitized regions of the cormorant alimentary canal were the proventriculus and gizzard but in very heavily infected birds, the nematodes were also detected in the esophagus. Examination of the histological sections of the bird alimentary canal revealed that nematodes were located deep in the gastric glands. This host-parasite interaction induced hyperplasia of the mucous cells and abundant mucus secretion around the body of the worm. In several sections, an inflammatory reaction was observed in the mucosal layer adjacent to the nematodes that consisted of focal accumulations of macrophages and mast cells. The same cells and collagen fibres were observed in the muscle layer of the infected organ. In birds, the physical and immunological environment of the gut can be changed by parasite infection resulting in a wide range

of interactions in the microbiota. In the lumen of most infected gastric glands, clusters of bacteria embedded in cellular debris were frequently observed. The occurrence of a very high number of bacteria near the nematode cuticle or laid upon it was documented. Such bacteria attached firmly to the *C. rudolphii* body by means of fimbriae or pili, such bristle-like protein appendages are observed on the surface of many bacteria, crucial for adhesion to host cells or surfaces. In previous histopathological papers on the cormorant-*Contracaecum* host-parasite relationship, bird immune cells documentation is absent. This paper provides an overview on the specific defence mechanisms of the great cormorant's digestive tract against *C. rudolphii*. Emphasis will be placed on the immune cellular response involving mast cells, macrophages and mucous cells against the nematode infection and novel observations on the interaction between bacteria and *C. rudolphii* will be provided.