Pathology associated with *Contracaecum rudolphii* (Nematoda: Anisakidae) infection in the great cormorant *Phalacrocorax carbo* (L. 1758)

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Summary

This is a report of lesions associated with the nematode *Contracaecum rudolphii* (Nematoda: Anisakidae) from the proventriculus of the great cormorant, *Phalacrocorax carbo* (L. 1758). The study was undertaken as part of a health monitoring program for *P. carbo*, which is endangered and thus protected within the European continent. Cormorants were collected by gun-shot from north-eastern Poland in the spring of 2006, four birds were necropsied on site and the gastrointestinal tract was examined for the presence of nematodes. The birds came from a region with noted increases in the cormorant population over the last decade. Esophageal and gastric sections with parasites in situ were fixed in formalin and processed routinely for paraffin embedding, stained with H&E and examined by brightfield microscopy. Parasite associated lesions consisted of severe, ulcerative gastritis at the attachment sites, and diffuse granulomatous gastritis in adjacent areas. Eosinophilic material speculated to be the parasite-derived excretory-secretory product was consistently forming the parasite-host boundary at the attachment points. Although the parasite-associated gastric lesions were focally severe, all examined birds appeared in good body condition. Because only four birds were investigated in this study, the potential contribution of *C. rudolphii* to morbidity and mortality in great cormorants needs to be examined further.

Keywords: great cormorant; *Contracaecum rudolphii*; parasitic gastritis; *Phalacrocorax carbo*; secretory product

Introduction

The great cormorant *Phalacrocorax carbo* (L. 1758) has the widest geographical distribution among the three cormorant species nesting in Europe. Its range includes the entire eastern hemisphere and the Atlantic coast of North America (Del Hoyo *et al.*, 1992). The European population of the great cormorant has increased from a few thou-

sand nesting pairs in 1980 to approximately 200 – 250 thousand pairs in the year 2000 (Bregnballe *et al.*, 2003). In Poland there was an estimated 14 % annual increase of the great cormorant population between 1981 – 1992, and currently there is an estimated 20 – 22 thousand nesting pairs in this country (Goc, 2006). This spectacular comeback of the cormorants can be attributed to strict protection measures, to changes in the ichthyofauna with increases in stocks of their prey i.e. middle sized fish predominantly in the families cyprinidae and percidae, and to the ban on agricultural use of DDT-containing pesticides. The above compound bioaccumulated along the trophic chain and led to marked thinning of eggshells resulting in decreased hatching success in birds (Dirksen *et al.*, 1995).

The great cormorant is a true piscivorous bird that feeds on numerous species of fish and thus is exposed to a variety of parasites that use fish as intermediate hosts. *Contracaecum rudolphii* is a cosmopolitan nematode that is frequently found in the digestive tract of piscivorous birds (Kuiken *et al.*, 1999; Torres *et al.*, 2000). This is also the most common proventricular nematode found in the great cormorant from northern Poland (Kanarek *et al.*, 2002). Very young birds can get infected by third stage larvae in their food (Kanarek *et al.*, 2002), and the prevalence of infection increases with the age of the birds (Kuiken *et al.*, 1999). Accordingly, studies of double-crested cormorants *Phalacrocorax auritus* from southeastern Canada, reported the prevalence of infection to be 50 % in one-week chicks, 83 % in two weeks old birds, and to reach 100 % in adult birds (Kuiken *et al.*, 1999). A similar pattern of prevalence has not been detected in cormorants from southern Germany and Switzerland (Nottenkämper *et al.*, 1999).

Most of the work on the parasites from cormorants concentrates on taxonomy of particular helminth groups like, nematodes (Nottenkämper *et al.*, 1999), or describes a wider spectrum of helminthes from cormorants (Kuiken *et al.*, 1999). Limited descriptions of pathology associated

with gastric infections with *C. rudolphii* and *C. multipapilatum* were reported in double crested cormorants from North America (Huizinga, 1971; Kuiken *et al.*, 1999) but no such reports exist for *C. rudolphii* infecting cormorants in Poland. The objective of our research was to describe gross and microscopic lesions from *P. carbo* infected with *C. rudolphii*.

Materials and methods

Twenty six cormorants were investigated for presence of *Contracaecum rudolphi*, four were examined for pathological changes. Birds were collected in April 2006 from a colony near Elk in north-eastern Poland (53°50'N, 22°25'E). The collection was done with the approval for scientific data acquisition issued by the Warmińsko-Mazurski Regional Authority (Permit # III6631-4-1/06).

The birds were collected by gun-shot by licensed hunters and necropsied on site. The gastrointestinal tracts were removed *in toto*, and then subdivided into the esophagus, proventriculus, ventriculus, and intestine which were cut longitudinally and examined for the presence of *C. rudolphii*. The nematodes collected from a particular segment were counted, measured and then fixed in 10 % buffered formalin. Full-thickness samples of the proventricular wall from infected birds were used for histopathology; three sampling sites, including regions with parasites *in situ* and free of worms, were collected from each proventriculus. After 48 hrs of fixation in 10 % buffered formalin, the samples were trimmed, processed routinely for paraffin embedding, sectioned at 4 µm, stained with hematoxylin and eosin (H&E), permanently mounted on glass slides using standard histological techniques, and examined by brightfield microscopy (Axioscopy FI - Carl Zeiss Opton, 1997).

Results

All infected birds were in excellent body condition, with well-developed musculature and moderate visceral fat. Five adult *C. rudolphii* were found free in the lumen of the esophagus in bird No. 3, and numerous adult nematodes were present in the proventriculi, either attached or free in the lumen, of all four birds (Table 1). Larval *C. rudolphii* were found in the proventricular lumen of birds No. 2, 3, and 4 (Fig. 1). The intestines had no parasites. The number, sex, and mean length of *C. rudolphii* from all birds are shown in Table 1.

Fig. 1. Adult *Contracaecum rudolphii* attached to the proventricular mucosa of a black cormorant *Phalacrocorax carbo*

In all birds examined, the adult nematodes attached within small approximately 0.2 cm, irregular, sunken areas of mucosa that was deeply red as compared to the normal tan-colored mucosal surface (Fig. 1). In addition, similar in character, but more tan foci without parasites, were found multifocally on the mucosa. Unfortunately, parasites detached during tissue processing and what follows is the description of microscopic mucosal lesions without parasites *in situ*. The attachment sites with adult nematodes observed macroscopically, were histologically characterized by deep mucosal ulcers overlying areas of necrosis and inflammation of variable severity and depth. The macroscopic tan foci without parasites were compatible with more chronic, healing ulcers. In many sections, the surface of the latter, was heavily colonized by a mixed bacterial flora. In such cases, numerous bacterial colonies were found along the luminal margin of a homogenous eosinophilic material that formed semicircular, crater-like structures with their luminal border conforming in shape to the anterior part of the nematode (Fig. 2 and 3). This conformation of shapes was further elaborated by areas of a deeply serrated border found only on the crater-like luminal surface of the eosinophilic material (Fig. 2). Similar, but smaller serrations were found on larval nematodes found free in proventricular lumen (insert, Fig. 3). The basal margin of the eosinophilic material was infiltrated by a mixed inflammatory cell population with heterophils, macrophages, plasma cells and lymphocytes. Because the size and position of the crater suggested that it was at some point the location of parasite attachment (Fig. 3), it is

Table 1. Gender, numbers, and lengths of adult and larval nematodes found in the examined birds

	No. of males	Mean length (mm)	No. of females	Mean length (mm)	No. of larvae	Mean length (mm)
1	8	299	30	372	none	-
2	15	284	30	356	15	153
3	18	273	47	345	20	155
4	17	273	25	362	5	148

Fig. 2. Proventricular mucosa with abandoned attachment site of *C. rudolphii*. Single arrows point to cross-sections of larval nematodes free in gastric lumen. There is marked bacterial colonization B, on the surface of the crater-like structure formed presumably by the secretory product SP, double arrows point to the focally serrated luminal border on SP. N denotes marked granulomatous inflammation effacing the mucosa M, and infiltrating into the submucosa.

highly plausible, that the eosinophilic material consisted of the secretory product SP of the nematode mixed with digested/necrotic host tissue. In support of this hypothesis, identical eosinophilic craters, but without bacterial colonization were also found in the sections that had adult parasites in situ (Fig. 4). These “current” attachment sites were surrounded by areas of acute or subacute hemorrhage with numerous hemolysed RBC and hemosiderin-loaded macrophages. The mucosa and submucosa subjacent to both, abandoned and “current” attachment sites had moderate to severe granulomatous inflammation expanding and

effacing the lamina propria and gastric glands (Fig. 2). In most sections the inflammation involved only the mucosa and submucosa, but in few, transmural inflammation expanded into the muscular layer with inflammatory cells found within endomysial connective tissue. Damage to the muscle was not observed in the sections examined. The inflammatory infiltrate was composed predominantly of lymphocytes and histiocytes, but focal accumulations of foreign-body type giant cells and diffusely scattered small numbers of heterophils and eosinophilic granulocytes were also present. In areas remote from parasite-attachment

Fig. 3. Higher magnification of area depicted in Fig. 2 showing crater-like attachment site SP, heavily colonized by bacteria B. Arrows point to the serrated luminal border of the SP (arrow on insert points to smaller ridges on larval nematodes). Note the necrosis and inflammation abutting the basal aspect of SP.

site, there was multifocal mild to moderate, transmural granulomatous gastritis and multifocal minimal mucosal hemorrhages.

Discussion

It is noteworthy that most sections had several ulcers clustered in one area which might suggest a grazing behavior exhibited by the parasite. Such grazing is most likely prompted by host immune defenses in the form of cell-mediated or humoral immunity products, which is supported by the lymphocytes and plasma cells found infiltrating the basal aspect of attachment sites. Our observations also suggest that bacterial colonization/infection of abandoned attachment sites might exaggerate the mucosal

damage and inflammation initiated by the parasite.

The role of *C. rudolphii* in morbidity and mortality of its host has been debated, and some authors (Owre, 1962) have postulated that a mutualistic relationship exists between the two. This has been suggested by observations that the movement of nematodes in proventricular lumen aided the bird's digestion by fragmentation and mixing of the ingesta. In contrast, Huizinga (1971) refuted such mutualism based on the fact that the absence of ingesta results in the attachment of the parasites to the proventricular wall and damage of host tissues. Accordingly, high mortality in pelicans *Pelecanus crispus* overwintering in Macedonia in 1991 – 93 has been associated with severe ulcerative and hemorrhagic gastritis due to infections with *Contracaecum* sp. coupled with lack of food caused by

Fig. 4. Proventricular mucosa with active attachment site of *C. rudolphii*, parasites detached during tissue processing. Note the SP with serrations (arrows) and lack of bacterial colonization; the mucosa surrounding the SP is totally effaced by necrotic and inflamed tissue and hemorrhage.

unusually cold winters (Pyrovetsi & Papazahariadou, 1995). Other authors support the parasitic rather than mutualistic relationship by reporting, that high intensity of infection with *C. rudolphii* interferes with digestion and absorption, and it usually does contribute to mortality in birds debilitated by other stressors like bacterial infections or environmental pollutants (Nottenkämper *et al.*, 1999; Abollo *et al.*, 2001).

Histological lesions reported in associated with *Contracaecum* sp. in cormorants included gastric ulceration with bacterial colonization, superficial granulomatous gastritis, and intraluminal and intraepithelial hemorrhage (Kuiken *et al.*, 1999; Huizinga, 1971). Although the above authors admitted that these lesions might lead to debilitation of the infected birds, no mortality was attributed to the parasites. Our results are in agreement with the above observations,

in that we found severe, ulcerative, granulomatous gastritis with multifocal hemorrhage to be associated with attachment sites of adult *C. rudolphii*. The inflammation was usually focal, but in some sections a transmural gastritis was present. In addition, we found multifocal granulomatous inflammation and hemorrhage in the mucosa and submucosa, not apparently associated with adult attachment site. The association of the latter lesions with *C. rudolphii* remains to be elucidated.

Lesions similar to the described above were reported from stomachs of definitive and intermediate hosts harboring the nematode *Anisakis simplex* (Young & Lowe, 1969), and from terrestrial mammals experimentally infected with anisakis larvae (Rokicki *et al.*, 1993). The tissue damage present in association with *A. simplex* is attributed mainly to its excretory-secretory products SP (Morris & Sakanari,

1994). The proteolytic enzymes contained in such secretions from many parasites including *A. simplex* have several functions. They aid in organ penetration by digesting the connective tissue matrix, provide an immunoprotective mechanism, are anticoagulants, facilitate larval molting, and play a major role in external digestion and nutrient uptake by the parasites (Knox & Kennedy, 1988; Moczoń & Wranicz, 1999). A similar role is played by other, non-proteolytic enzymes within the SP, including hyaluronidases (Hotez *et al.*, 1994), leucine-aminopeptidase (Rhoads *et al.*, 1997), acetylcholinesterases (Opperman & Chang, 1992) and other hydrolases (Dziekońska-Rynko *et al.*, 2003). Studies by Dziekońska-Rynko and Rokicki (2005) failed to document the presence of endoproteases in the SP products from adult *C. rudolphi*, but high levels of leucine-aminopeptidase, and different esterases and glycosidases were detected. The latter enzymes can be responsible for damage of host tissues and it has been shown that they play a role in tissue penetration and nutrient uptake during parasite migration within the respiratory and digestive tract of their hosts (Irwin *et al.*, 2004). The proventricular lesions reported in our study as well as by others (Huizing, 1971; Kuiken *et al.*, 1999) are possibly caused by the tissue-degrading enzymes secreted within SP products of *C. rudolphi*, because the morphology of its mouth apparatus does not suggest its participation in tissue damage. In summary, this paper is the first report of histopathological lesions associated with infection of the great cormorant *Phalacrocorax carbo* (L. 1758) with adult and larval *C. rudolphi* in a population of birds from north-eastern Poland. The health effects of this nematodiasis on the health status of wild cormorants need to be established in future studies.

Acknowledgement

This work was supported by the Ministry of Education and Science grant No. 0420/PO4/2005/28.

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RECEIVED FEBRUARY 19, 2009

ACCEPTED NOVEMBER 12, 2010