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Intestinal Histopathology due to an Acanthocephalan in Two Corvid Species from Northern Italy

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ABSTRACT: Carnivorous birds maintain parasites in the sylvatic cycle and have a role in their diffusion. The histopathology and ultrastructure of the intestine of 29 Hooded Crows (*Corvus corone cornix*) and 51 Eurasian Magpies (*Pica pica*), from the Province of Ferrara (Northern Italy), naturally infected with *Sphaerirostris picae* (Acanthocephala), were investigated. In both bird species, the prevalence of infection was around 10%, and the intensity of the infection in the Hooded Crows ranged from two to 12 acanthocephalans per host, whereas in the Eurasian Magpies it ranged from one to nine worms per bird. Previous records on the histopathology of acanthocephalans in birds do not provide information on the type of cells involved in the host's reaction. We aimed to gain information on the effects of acanthocephalans on the structural integrity of the birds' intestine and to describe the type of immune cells in the hosts against the parasite. Our results showed that *S. picae* disrupted the intestinal wall at the site of attachment by means of its neck and proboscis, and three main types of bird intestinal reactions were noticed. The most severe response of the hosts was against the proboscis because of the action of its hooks with recruitment of macrophages, giant cells, eosinophils, and heterophils. *Sphaerirostris picae* perforated the birds' entire intestinal wall, reaching the peritoneal visceral serosa, but it did not provoke a diffuse peritonitis.

Key words: Digestive tract, histopathology, *Sphaerirostris picae*, wild birds.

The Corvidae (Passeriformes) belong to the most developed avian group. There is no information on the occurrence of enteric helminths in corvids in Northern Italy, nor on their histopathologic effects on the host's digestive tract. We studied the histopathology caused by an enteric helminth, *Sphaerirostris picae* (Acanthocephala) in the Hooded Crow (*Corvus corone cornix*) and the Magpie (*Pica pica*).

A protocol was developed to conduct a study in the Province of Ferrara (Emilia-Romagna region, Northern Italy) on Hooded Crows and Eurasian Magpies, including the mobilization of federal hunting guards or veterinarians and the organization for the collection and transport of healthy and moribund birds to the Experimental Zooprophylactic Institute (Ferrara Branch). The plan was established during one hunting season, between September 2015 and November 2015. In about 3 mo, 29 Hooded Crows and 51 Eurasian Magpies were provided to the Institute.

Birds were captured in Larsen traps and euthanized by means of cervical dislocation according to the provisions set out by the national and European legislation on animal welfare (European Parliament and Council 2010). After the dissection of the bird, the whole digestive tract and associated organs were removed from the body and searched for the presence of helminths. In both bird species, acanthocephalans were found still attached to the intestine. The position was recorded, and 15×15 mm pieces of the intestine were fixed in 10% neutral buffered formalin for 24 h for light microscopy. Additionally, 7×7 mm pieces of intestine were excised and fixed in chilled 2.5% glutaraldehyde for 3 h for electron microscopy Dezfuli et al. (2015).

The prevalence of *S. picae* was 10.3% (3/29) in Hooded Crows and 9.80% (5/51) in Eurasian Magpies, and the intensity of the infection varied from two to 12 worms for the Hooded Crows and one to nine worms for the Eurasian Magpies. This worm induces changes in the structural integrity of the hosts' intestines. Both male and female acantho-

cephalans penetrated deeply through all layers of the host intestines. We found three main types of tissue reactions at the site of parasite attachment. The first response was parasite metasoma in the gut lumen consisting of the desquamation of the apices of the intestinal villi (Fig. 1A) and hyperplasia of mucous cells with prevalent acid mucin (sialomucin) secretion near the point of attachment. The intestinal lamina propria was congested or hemorrhagic from an infiltration of eosinophil granulocytes; the recruitment of this cell type was noticed also in the intestinal epithelium above the granular cells bearing large ovoidal eosinophilic granules (Fig. 1B). Lymphocytes and heterophils were also encountered in the lamina propria. The histopathologic pattern was consistent with eroding, catarrhal, eosinophilic enteritis. The second type of response was a reaction induced by the neck of the acanthocephalan within the intestinal wall that disrupted the mucosa, the submucosa, and the muscularis layer of the intestine (Fig. 1C). Proliferation of the lamina propria and submucosa, congestion, hemorrhage, infiltration of lymphocytes and eosinophils and fibroblastic-connective reaction were observed (Fig. 1D). The third type of reaction was the most severe response against the proboscis from the action of its hooks. In this reaction, the muscle layer was substituted with thick fibroblastic-connective tissue (Fig. 1E). Besides lymphocyte and eosinophil granulocytes, macrophages and foreign body giant cells that occurred near the focal accumulation of amorphous azurophilic proteinaceous material, recruitment of macrophages, and giant cells were the most notable reactions to the proboscis (Fig. 1D, F). Numerous acanthocephalans perforated the entire intestinal wall, provoking an intense fibroblastic-connective tissue reaction. A deep penetration of the proboscis reached the peritoneal visceral serosa (Fig. 1C, E, F) but did not produce a diffuse peritonitis. Transmission electron microscopy revealed the feature of the cells involved in the inflammatory reaction (Fig. 2A). Particular attention was paid to eosinophils and heterophils (Fig. 2B) because it was not easy to make an accurate distinction between the cells by light microscopy. Eosinophils showed large ovoidal granules (Fig. 2C) clearly distinguishable from the smaller, heterogeneous, spindle-shaped granules of heterophils (Fig. 2D). Degranulation and granule dissolution near the proboscis occurred frequently (Fig. 2E).

In most bird species, acanthocephalans perforate the alimentary canal of its hosts, as documented in this study of the Hooded Crow and the Eurasian Magpie, but we found no signs of diffuse peritonitis. Indeed, during the present survey, a local chronic encapsulating granulomatous, fibroconnective tissue reaction occurred. A similar reaction was reported in cormorants infected with the acanthocephalan *Southwellina hispida* (Dezfuli et al. 2002).

Several accounts describe the presence of acanthocephalans in wild birds (e.g., Dezfuli et al. 2002; Santoro et al. 2010), including corvids (Halajian et al. 2011; Radwan 2012; Khatoon et al. 2014). Nevertheless, previous histopathologic records on corvids (Andrews and Threlfall 1975; Halajian et al. 2011; Khatoon et al. 2014) did not provide detailed information on the types of cells involved in the host reaction, thus any comparison with our data is not possible. Inflammation of the mucosa, with infiltration by heterophils and macrophages, was reported for some raptors infected with an acanthocephalan (Santoro et al. 2010). In this study, besides the chronic granulomatous reaction and the fibroconnective encapsulation, the occurrence of eosinophils was the main reactive feature. In mammals, the role of eosinophils against parasite infection is well known (Montali 1988), whereas birds have contrasting findings on eosinophil recruitment at the site of parasite infection (Davison et al. 2008; Claver and Quaglia 2009; Klion et al. 2020).

A zebrafish (*Danio rerio*) model shows an evolutionarily conserved response of eosinophils to allergens and parasites among vertebrates (Balla et al. 2010). Avian eosinophils might not easily be distinguished from heterophils. Indeed, eosinophils do not display a typical shape of eosinophilic granules in all avian species (Clark et al. 2009), which is

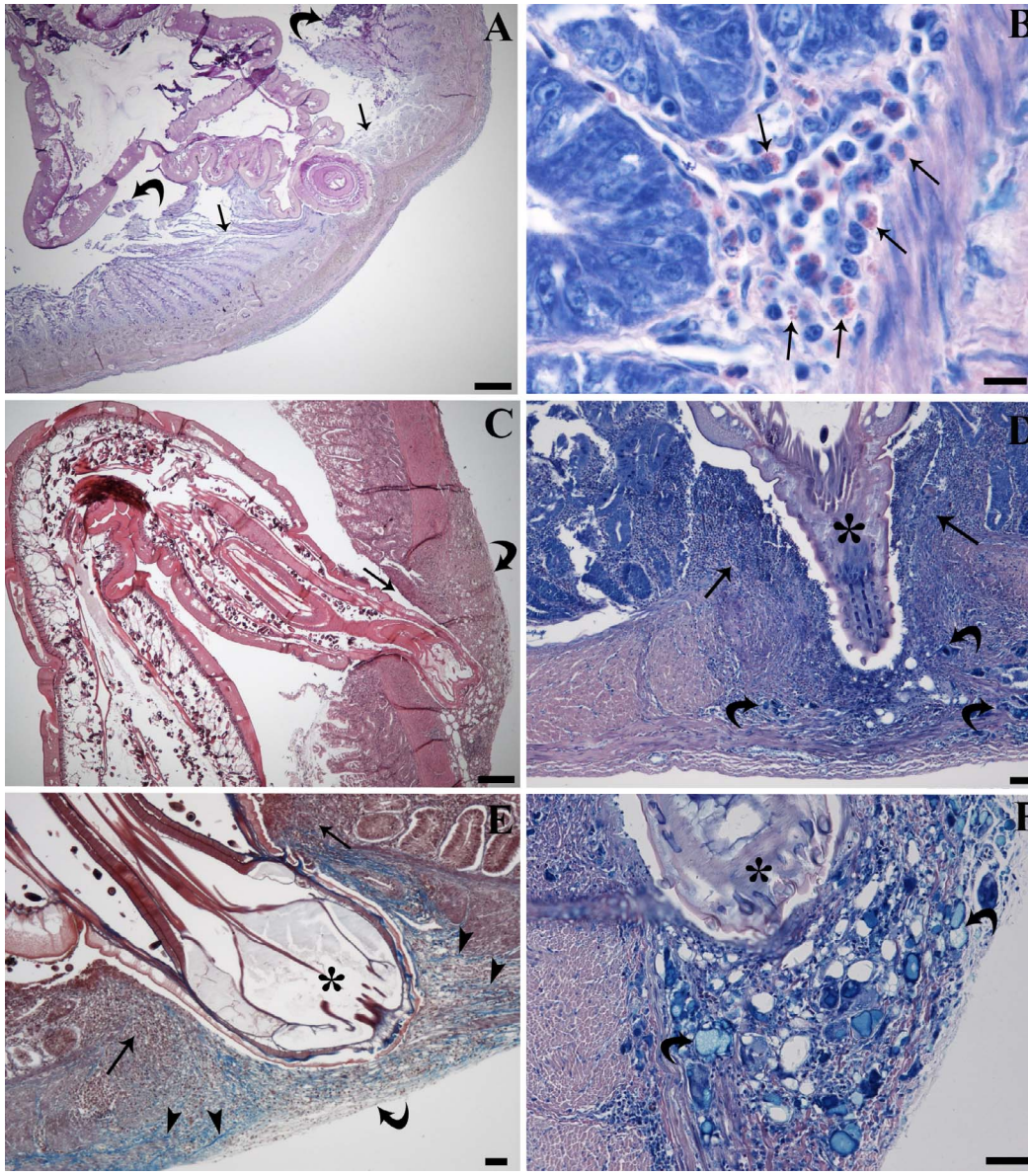

FIGURE 1. Histologic sections of the intestine of Hooded Crows (*Corvus corone cornix*) and Eurasian Magpies (*Pica pica*) infected with *Sphaerirostris picae*. (A) Sagittal section of the intestine of Hooded Crows with an attached female *S. picae*. Note the destruction of the apex of intestinal folds (arrows) near the parasite trunk. Presence of catarrhal enteritis (curved arrows) within the intestine lumen is evident. Alcian/periodic acid–Schiff stain. Bar=200 μm. (B) Sagittal section of the intestine of Hooded Crows; within the lamina propria, eosinophil granulocyte (arrows) infiltration occurs. Giemsa stain. Bar=10 μm. (C) Micrograph shows how the female *S. picae* with neck and everted proboscis disrupted the intestinal layers (arrow) of the magpies. Note the depth of the proboscis penetration in the peritoneal visceral serosa (curved arrow). H&E stain. Bar=200 μm. (D) Penetration of the proboscis (asterisk) within the submucosa and muscularis layers of the Eurasian Magpie intestine is visible. Note the infiltration of granulocytes (arrows) and the accumulation of amorphous azurophilic material and giant cells (curved arrows) around the proboscis. Giemsa stain. Bar=50 μm. (E) Deep penetration of the proboscis (asterisk) in the peritoneal visceral serosa (curved arrow) of the Hooded Crows. The presence of numerous granulocytes (arrows) and fibroblastic-connective tissue (arrowheads) is evident. Masson's trichrome stain. Bar=50 μm. (F) The proboscis (asterisk) is embedded in the peritoneal visceral serosa of the Eurasian Magpie intestine. Recruitment of amorphous azurophilic material (curved arrows), macrophages, and giant cells below the proboscis can be seen. Giemsa stain. Bar=50 μm.

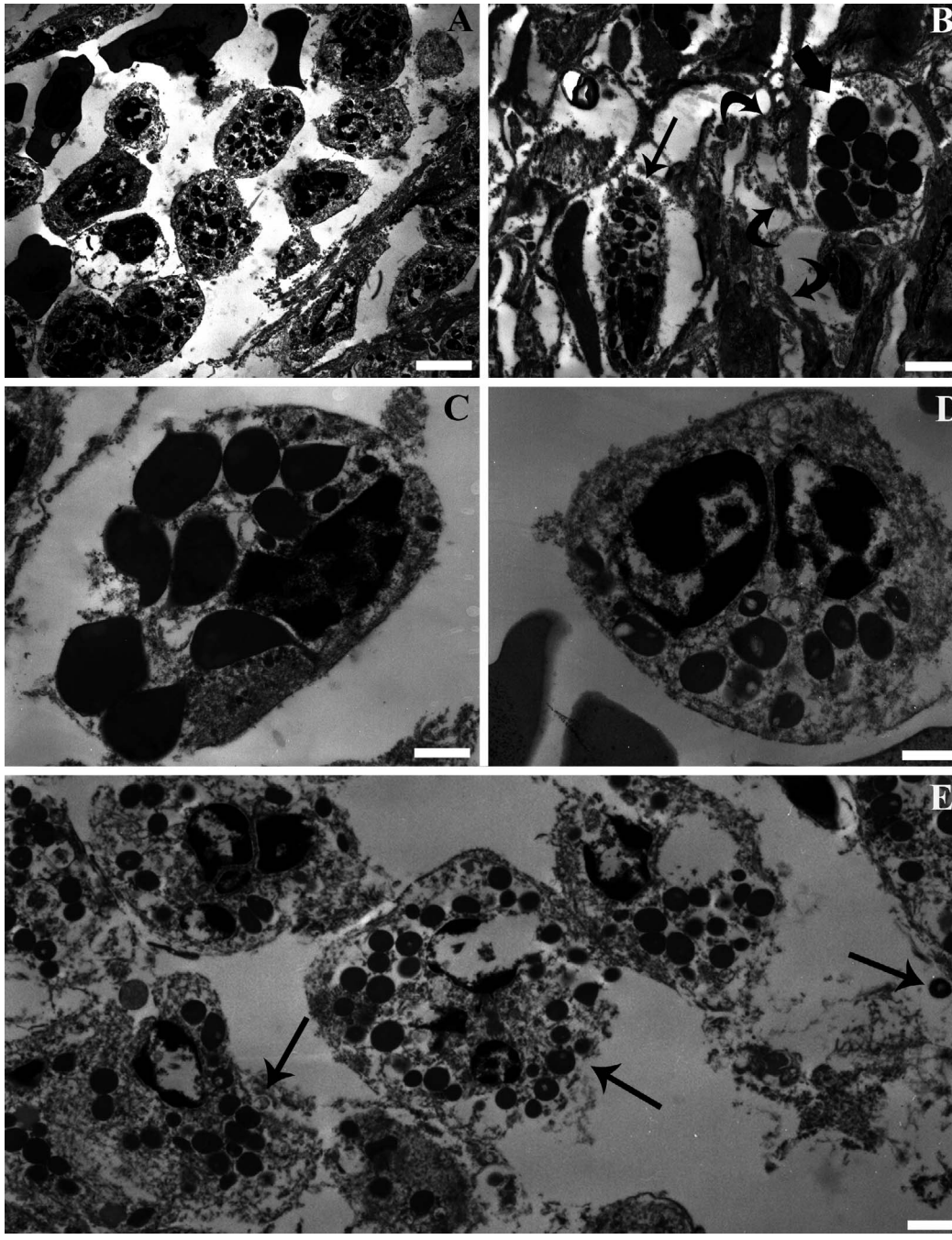

FIGURE 2. Electron micrographs of the infected intestine of Hooded Crows (*Corvus corone cornix*) and Eurasian Magpies (*Pica pica*). (A) Micrograph of the infected intestine of Hooded Crow. Several granulocytes inside a vessel near the parasite's proboscis are visible. Bar=2.8 μm. (B) Parasitized intestine of Hooded Crows. An eosinophil granulocyte (arrow) and heterophil (thick arrow) are separated by collagenous fibers (curved arrows). Note the difference between the size and shape of electron-dense granules of two cells. Bar=1.6 μm. (C) Infected intestine of Eurasian Magpies. High magnification of an eosinophil showing the eccentric nucleus and the cytoplasm filled with some big electron-dense granules. Bar=0.6 μm. (D) Intestine of magpies. The micrograph shows a heterophil with eccentric nucleus and several spindle-shaped, electron-dense granules. Bar=0.6 μm. (E) Parasitized intestine of magpies. The section in close proximity to the acanthocephalan's proboscis and degranulation of some granulocytes (arrows) are visible. Bar=1.2 μm.

presumably the reason for the lack of uniformity in identifying the above cells in histopathologic accounts in birds. Nevertheless, even the role of mammalian eosinophils needs to be revised because most functions are still unclear or poorly described (Simon et al. 2020).

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