Sci Parasitol 16(4):151-155, December 2015
ISSN 1582-1366

ORIGINAL RESEARCH ARTICLE

Histopathology of *Centrorhynchus globirostris* (Acanthocephala: Centrorhynchidae) infecting the intestine of the pheasant crow, *Centropus sinensis* (Stephens) in Pakistan

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Abstract. The tissue pathology of the intestinal lining of a pheasant crow *(Centropus sinensis)* invaded by an acanthocephalan *(Centrorhynchus globirostris)* was studied from prepared slides. Prominent histopathology was evident viewing the infected tissue. There is extensive damage to the intestinal layers of the pheasant crow initiated by the multi hooked proboscis of the worm. The worm would often work its way into the outer smooth muscle layers of the intestine (muscularis externa) causing host cell necrosis, hemorrhaging and obstruction of the inner mucosal layer. Results of the worm invasion included necrosis of the host epithelial cells, blood loss with pools of nucleated red cells and granular cells outside the capillary walls, and decreased potential for host absorption of nutrient products. An invasion of this worm would definitely affect the absorption capabilities of necessary metabolic products for host life.

Keywords: Pathology; Acanthocephala; Pheasant Crow; *Centrorhynchus globirostris*.

Received 22/09/2015. Accepted 08/11/2015.

Introduction

Centrorhynchus globirostris is a new species of Acanthocephala infecting the pheasant crow *(Centropus sinensis)* which was recently described (Amin et al., 2015). This acanthocephalan has a long cylindrical trunk with anterior dilation, and transverse anastomoses of the secondary lacunar vessels and other unique characteristics (Amin et al., 2015). The host, pheasant crow, is a member of the Order Cuculiformes common in Pakistan and other Asian countries (Roberts, 1991).

The authors have included histopathology in previously-published studies (Heckmann and Halajian, 2012; Amin et al., 2011; Amin et al., 2012; Heckmann et al., 2014). The understanding of the host tissue response is an important aspect for the biology of the infected host. The objective of this study was to understand and evaluate the host response (pheasant crow) of the invasive

acanthocephalan *C. globirostris.* It is our goal to show the effect the worm has on the intestinal lining of the host.

This is the first published study of the histopathology of *C. globirostris* infecting the pheasant crow *(C. sinensis)* from Pakistan.

Materials and methods

Infected host **intestinal** tissue was fixed in 10% buffered formalin and stored in 70% ethanol. Standard methods were used for slide preparation including dehydration, clearing and blocking (Bancroft and Gamble, 2001; Kienan, 2002). The paraffin blocked tissue was sectioned at 4-6 µm using a Leica Model RM 2255 Microtome, placed on glass slides and stained with hemotoxylin and eosin (H&E) in an automated tissue processor. Additional sections were stained with Mallory's trichrome to emphasize pathological responses to the parasite (Galigher and Kozloff, 1971). The prepared glass slides were viewed with a Zeiss Axovert 135 Inverted Compound Light Microscope with a Pentax K100 (digital imaging) Camera attached. Representative pictures were taken for future reference at varying magnifications and stored on a USB. H&E is a standard stain for tissue whereas Mallory's trichrome helps differentiate granular and necrotic tissue typical of parasite invasion.

Results

Histopathology

Sections of the infected intestine of the pheasant crow had worms firmly attached to the inner mucosal lining. These acanthocephalans, *Centrorhynchus globirostris,* were relatively large for the host intestine causing visible damage to the layers of tissue. Figures 1 to 6 are the results of the examination of the infected tissue. The results are typical for the tissue invasion of an acanthocephalan worm. The normal tissue lining of the avian intestine is represented by figure 1 showing typically columnar epithelial-lined crypts near the smooth muscle fibers of the muscularis externa. *C. globirostris* attaches to the outer mucosa of the lumen with the aid of the well-armed proboscis causing subsequent tissue necrosis, hemorrhaging and macrophage migration (figures 2 and 3). These later 2 figures show the proboscis next to the outer smooth muscle layer of the intestinal submucosa. Proboscis hooks, used for host attachment, are visible. Figure 2 displays the pathway of the proboscis into the host intestine. The worm may release its attachment leaving a prominent cavity with surrounding host necrotic tissue with some visible connective tissue encapsulation and smooth muscle damage (figures 3 and 4). Figures 4 and 5 represent higher magnification of the damaged host tissue within the inner lining of the intestine. Extensive hemorrhaging due to damaged capillaries has occurred with tissue necrosis. Note the damaged smooth myofibers next to the host submucosa. Connective tissue fibers are also visible which are involved with encapsulation of the worm (figure 4). Compaction of villi with resultant necrosis and lose of the typical mucosal surface is common for the invaded tissue (figures 2 and 3).

Numerous nucleated red blood cells and granulocytes can be observed surrounding the host mucosa where the worm has attached. Due to the size of the worm and the attachment to the mucosal lining the absorbing surface of the host intestine is not only damaged but also obstructed for potential nutritional capabilities (figures 3 and 4). These observations have been reported in other articles for histopathology (Bullock, 1963; Amin et al., 2012). Hooks, the prominent attachment structure, are visible for the prepared tissue sections typical of Acanthocephala (figures 2 and 3). Evidence of attempted host encapsulation of the worm is visible for figure 4. The other slide sections (110 glass slides examined) displayed invasion of the host tissue with hemorrhaging and cell necrosis and disorganization. Both male and female worms were observed. Figure 5 shows a female worm within the body cavity after migrating through the host muscle wall. The male *C. globirostris* is shown in figure 6 with a prominent bursa and tegument lining.

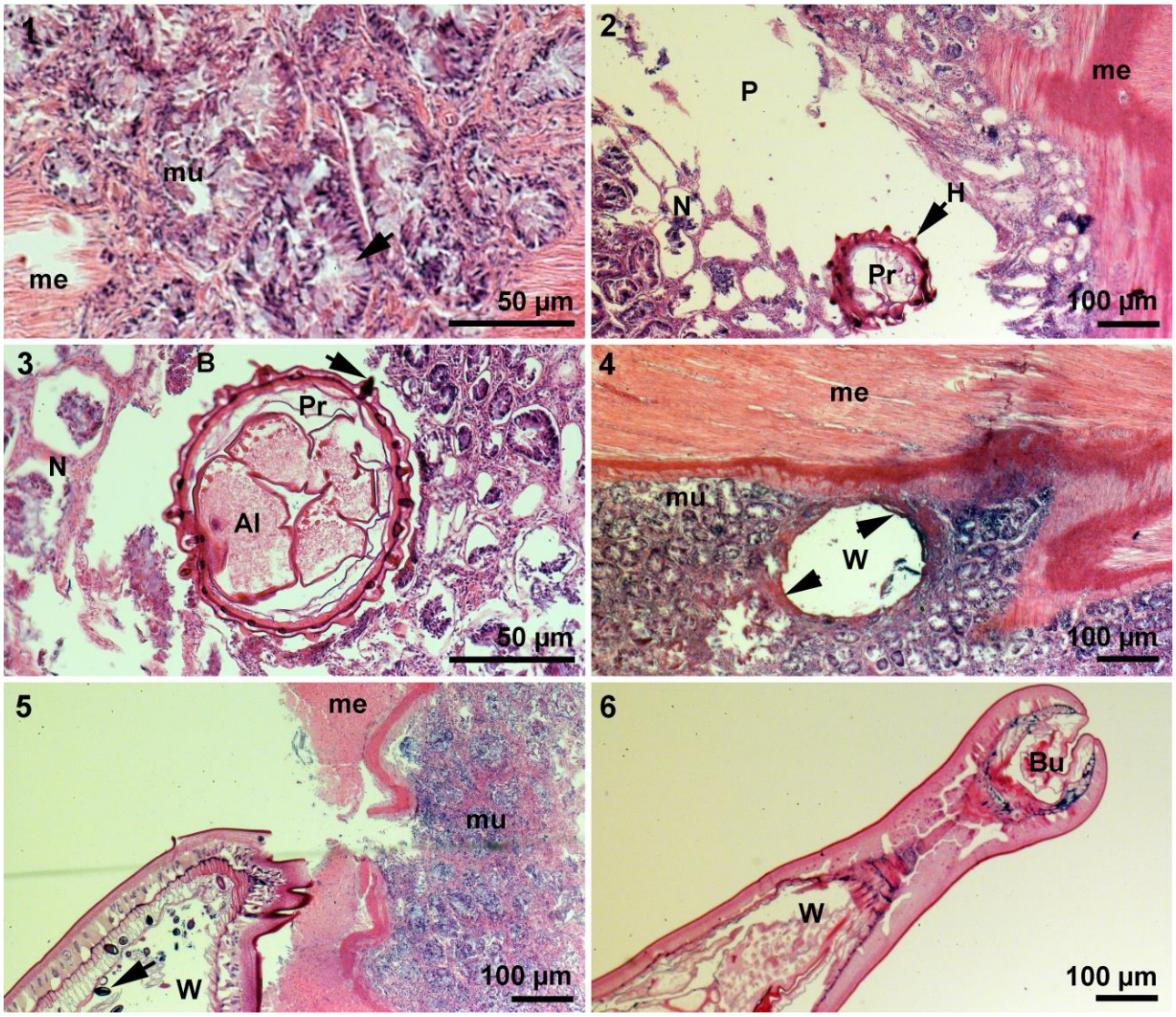

Figure 1. Represents normal mucosal (mu) tissue next to the muscularis externa (me) of the host intestinal tissue. Note the normal columnar epithelial cells (arrows) lining the lumen of the intestinal glands. All figures have been stained with H. & E.

Figure 2. The host tissue (pheasant crow) is invaded by the worm *(Centrorhynchus globirostris)* depicted by the penetrating proboscis (Pr) armed with hooks (H) showing the pathway (P) of the acanthocephalan. Note: (Me) muscularis externa, (N) necrotic host tissue and hemorrhaging surrounding the proboscis (Pr.)

Figure 3. Section of the invaded tissue at the higher magnification. Note the alveolar sacs (Al) inside the armed (Arrows) proboscis (Pr). Necrosis (N) and hemorrhaging (B) surround the proboscis.

Figure 4. Depicts the previous location of the worm (W) within the intestinal mucosa (mu) surrounded by the smooth muscle muscularis externa (me). The host has started to deposit collagenous connective tissue (arrows) before the acanthocephalan vacated the area. Extensive necrosis and muscle damage is visible with hemorrhaging.

Figure 5. A female *C. globirostris* (W) containing eggs (arrow) next to the damaged muscularis externa (me) and the necrotic mucosa (mu).

Figure 6. A male worm (W) found in the host intestine with a well pronounced bursa (Bu). Both male and female worms were prominent on all the tissue slides.

Discussion

A study of 110 intestinal tissue slides, prepared for light microscopy from an infected pheasant crow *(Centropus sinensis)* displayed classic histopathology due to the acanthocephalan *Centrorhynchus globirostris.* Histopathology is a well-used method for understanding the pathology of an invading worm (Venard and Warfel, 1953; Bullock, 1963; Whitfield, 1979; Mohamood et al., 2012).

The pheasant crow is a common bird in Pakistan (Roberts, 1991), along with other suitable avian hosts in the geographical area, that have been recorded as hosts for intestinal parasites. Tissue studies, especially the histopathology, is a valuable technique for understanding biological problems for all members of the animal kingdom (Heckmann, 2009; Heckmann et al., 2014). It is especially valuable for the study of fish parasites and gaining an understanding of the problems for the piscine host (Heckmann, 2013; Venard and Warfel, 1953). **As mentioned histology has been an important part of our past research projects.**

For *C. globirostris,* a well-armed proboscis attaches to the intestinal mucosa damaging the epithelial lining and causing capillary damage with subsequent blood lose and macrophage migration **including granular white blood cells.** The worms obstruct and compact the villi and mucosal lining of the intestine limiting the hosts absorptive surface. Both male and female worms were present increasing the potential parasite load for the pheasant crow and other potential hosts.

Acknowledgments

The authors wish to thank the Histology Laboratory (Mike Downey, Director) at the Provo Utah Valley Regional Medical Center for histopathology slide preparation. Provo, Utah. Kyrie Carpenter, Electron Optics Lab, BYU, helped prepare the plates for the figures with Michael Standing.

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