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Pulmonary Lesions caused by the Nematode *Rhabdias fuscovenosa* in a Grass Snake, *Natrix natrix*

Andrei D. Mihalca,[1,4] Viorel Micläuş,[2] and Menelaos Lefkaditis[3] [1] Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, Cluj-Napoca, 400372, Romania; [2] Department of Histology, Faculty of Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, Cluj-Napoca, 400372, Romania; [3] Laboratory of Parasitology, University of Thessaly, Veterinary Faculty, PO Box 199, Karditsa, 43100, Greece; [4] Corresponding author (email: amihalca@usamvcluj.ro)

ABSTRACT: In June 2005, a free-ranging grass snake, *Natrix natrix* infected with *Rhabdias fuscovenosa* was collected for histologic examination of the lungs. Gross lesions were not noted; however, histologic examination revealed vacuolar degeneration of the respiratory epithelium, hemorrhage, smooth muscle degeneration, faveolae necrosis and obstruction, and intralesional nematodes. Eosinophils were the most common inflammatory cell observed in the areas of necrosis and interstitium. Herein we describe histopathologic changes due to *R. fuscovenosa* in a free-ranging grass snake.

Key words: Lesions, *Natrix natrix*, *Rhabdias*, Romania.

Nematodes of the genus *Rhabdias* are common lung parasites of amphibians and reptiles (Anderson, 2000), with over 40 species described worldwide (Kuzmin et al., 2003). *Rhabdias fuscovenosa* (Railliet) is one of the most commonly reported nematodes parasitizing Palearctic and Nearctic snakes (Sharpilo, 1976; Baker, 1987; Kuzmin et al., 2003). Sporadic reports of *R. fuscovenosa* from African snakes (Hering-Hagenbeck and Boomker, 2000) and reports from lizards (Borkovcová and Kopřiva, 2005) are probably misidentifications of congeneric parasitic nematodes.

Nematodes of the genus *Rhabdias* may exist either in a free-living or parasitic stage. Only parthenogenetic females are parasitic; males are free-living. The life cycle is direct and no intermediate hosts are required. Infective larvae may enter the host via skin penetration or via penetration of oral mucosa after ingestion in food or water. After penetration, larvae gain access to the circulatory system and are distributed to the lungs, where they mature. Adult females produce embryonated eggs, which are shed through the feces of infected hosts (Anderson, 2000). Infection of snakes can occur without parasites going through the free-living stage (Jacobson, 2007).

Little information has been published on the histopathologic changes associated with *R. fuscovenosa* infection in free-ranging snakes. Results from laboratory studies on two snake species (*Nerodia sipedon* and *Thamnophis sirtalis*; Chu, 1936) and captive reptiles (Jacobson, 2007) infected with *R. fuscovenosa* have been reported, but no reports are available from free-ranging snakes. Herein, we report the results of gross morphologic and histologic examination of *R. fuscovenosa* infection in a free-ranging grass snake, *Natrix natrix*.

In June 2005, a free-ranging grass snake was found injured on a road near the city of Cluj-Napoca (46°43′36″N, 23°34′51″E), Romania. The animal was collected and humanely euthanized by intraperitoneal injection of 50 mg/kg sodium pentobarbital. Complete parasitologic examination was performed and epidemiologic results were published (Mihalca et al., 2007). Examination of lungs by binocular microscope revealed the presence of 34 nematodes ~3–5 mm in length (Fig. 1), subsequently identified as *R. fuscovenosa*. For histologic processing, lungs were fixed in 10% formalin and embedded in paraffin blocks; the blocks were serially sectioned at 5 μm. Lung sections were stained using Goldner's trichromic method for examination under light microscopy. Lesions were photographed using an Olympus DP10 camera attached to an Olympus BX41 microscope (Olympus Optical Co., Tokyo, Japan).

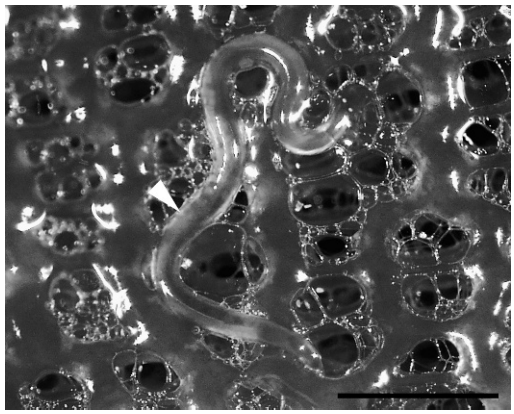

FIGURE 1. Adult *Rhabdias fuscovenosa* (arrowhead) surrounded by mucus in the lungs of a grass snake, *Natrix natrix*. Bar=1 mm.

Gross changes were not observed in the lungs; however, histopathologic changes were observed and were associated with the nematodes (Fig. 2). The respiratory epithelium had vacuolar degeneration and cellular debris filled the faveolae (Fig. 3). Other changes included intrafaveolar hemorrhage (Fig. 4), smooth muscle degeneration in the interfaveolar septa (Fig. 5), collapse of pulmonary structures, and obstruction of faveolae (Fig. 6). Inflammatory cell infiltrates were present within the faveolar lumen and interstitium and were predominated by eosinophils. Histologic examination did not reveal other causes for the pulmonary lesions and lesions were seen only in areas of the lung where nematodes were present.

The few reports on the pathology associated with *Rhabdias* infection in reptiles are from captive animals. In pet snakes there are reports of clinical disease including signs of hypoxia and severe pneumonia, with secondary bacterial pneumonia. Gross appearance of the lesions in these snakes consisted of accumulation of mucus around the nares

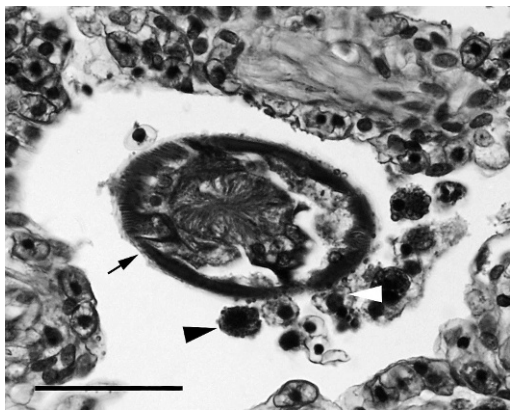

FIGURE 2. Faveolar cross section of lung in a grass snake, *Natrix natrix*, showing the presence of nematode (black arrow) surrounded by epithelial debris (white arrowhead) and eosinophils (black arrowhead). H&E stain. Bar=100 μm.

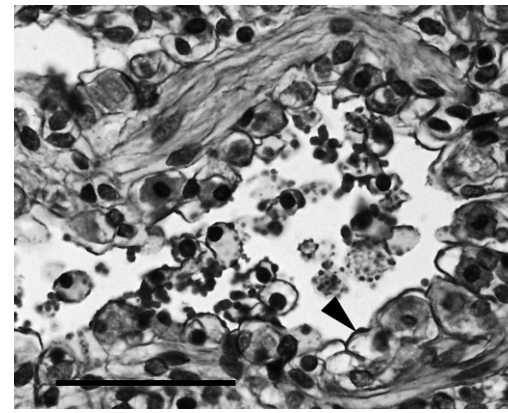

FIGURE 3. Faveolar cross section of lung in grass snake, *Natrix natrix*, with vacuolar degeneration of epithelial cells (arrowhead). H&E stain. Bar=100 μm.

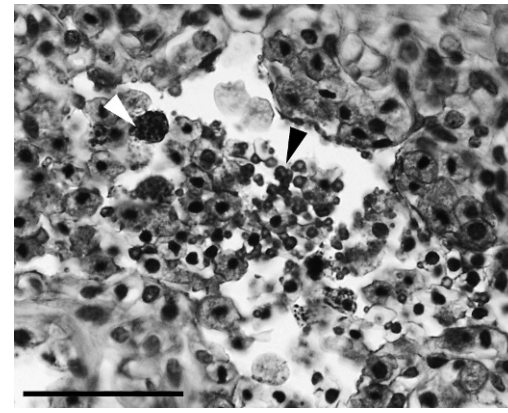

FIGURE 4. Cross section of lung in a grass snake, *Natrix natrix*, showing intrafaveolar hemorrhage (black arrowhead) with eosinophil infiltration (white arrowhead). H&E stain. Bar=100 μm.

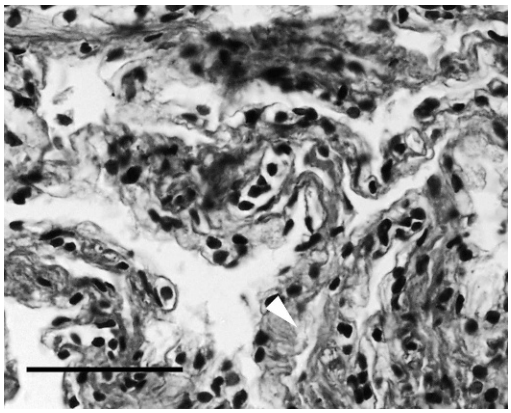

FIGURE 5. Cross section of lung in a grass snake, *Natrix natrix*, showing degenerated muscles (arrowhead) from the interfaveolar septa. H&E stain. Bar=100 μm.

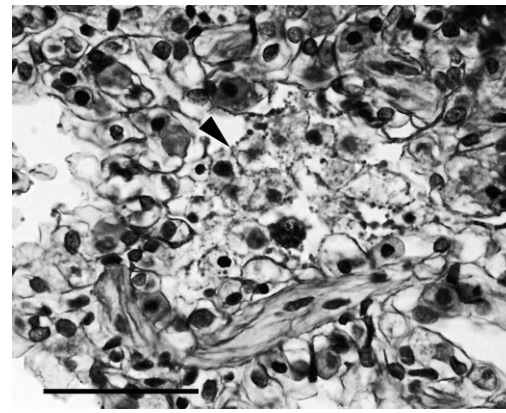

FIGURE 6. Cross section of lung in a grass snake, *Natrix natrix* showing faveolar obstruction (arrowhead) caused by destruction of epithelial layer. H&E stain. Bar=100 μm.

and glottis and necrotic cellular debris and exudate in the major air passageways (Jacobson, 2007). In experimentally infected snakes, Chu (1936) found no gross lesions produced by penetration of adult *Rhabdias* into the lungs in heavy infections; however, histopathologic changes were not described. Histologic changes described by Jacobson (2007) in captive snakes infected with *Rhabdias* consisting of proliferative pneumonia with hypertrophy of pneumocytes are similar to our findings. Jacobson (2007) and Schaftenaar et al. (2000) reported changes associated with the migration of larval nematodes of the genus *Rhabdias* through various tissues of lizards.

Our findings suggest that in free-ranging snakes, although infection with *Rhabdias* may not be clinically evident or cause gross lesions, histopathologic changes may be focally severe. Free-ranging reptiles are infected with a variety of parasites, but few reports associate parasitism with morbidity or mortality. Moreover, parasite-induced gross or microscopic lesions are commonly reported in free-ranging reptiles. For an extensive review, see Jacobson (2007). However, individual reptiles that become ill and die in the field are rarely found, unless there is an epidemic die-off (Jacobson, 2007). The impact of focal lesions on the general health of hosts is difficult to evaluate. In toads infected with *Rhabdias bufonis*, Goater and Ward (1992) showed parasite-induced anorexia with reduced growth and survival. No similar effects were reported in snakes infected with *Rhabdias*, but pulmonary lesions could impair oxygen consumption, as shown by Joly et al. (2008) in toads infested with pulmonary helminths.

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