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Pathomorphological studies on wild boars infected with *Metastrongylus* spp., *Ascarops strongylina*, and *Macracanthorhynchus hirudinaceus*

Mariana Panayotova-Pencheva, Katerina Todorova, Vassilena Dakova

Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Science, Sofia – 1113, Bulgaria
marianasp@abv.bg

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Abstract

Introduction: Pathomorphological changes in the lungs, stomach, and small intestines of wild boars infected with *Metastrongylus* spp., *Ascarops strongylina*, and *Macracanthorhynchus hirudinaceus* were investigated. **Material and Methods:** Dissection of 11 wild boars was performed, and parasitised organs were histologically investigated by common techniques. **Results:** Macroscopic lesions in the lungs infected with *Metastrongyus* spp. were seen within the apical parts of the large lobes, irregular in form, pale greyish in colour, and compact in consistency. The main pathohistological findings were: the presence of parasite forms, and lymphocytes and neutrophils in the lumen of bronchi and bronchioles, desquamation of the bronchial and bronchiolar epithelium, emphysema, thickening of alveolar septa, hyperaemia, alveolitis, infiltration of the interstitial tissue with giant cell, monocytes and eosinophils, and peribronchial and disseminated lymphoid hyperplasia. The principal observations accompanying infection with *A. strongylina* were inflammation and focal mucosal damage in the stomach, the latter clearly demarcated from the surrounding tissues. Severe injuries in the place of attachment of *M. hirudinaceus* to the wall of the small intestine were seen. Intestinal villi, underlying mucosa, and submucosa were destroyed, and an intense inflammatory reaction was present. **Conclusion:** The histopathological lesions showed wide diversity, varying from mild to severe; but none of them were lethal.

Keywords: wild boar, *Metastrongylus* spp., *Ascarops strongylina*, *Macracanthorhynchus hirudinaceus,* histopathological lesions.

Introduction

As an omnivorous animal, the wild boar is a subject to a large number of parasitic infections. Wild boar parasite species are the same as in domestic pigs. Gastrointestinal and pulmonary helminths are among the most common parasitic agents in swine. A number of studies show their negative influence on animal health and condition. Knecht *et al.* (4) found experimentally that the level of gastro-intestinal parasite infection negatively affected pig carcass meat content. Patra *et al.* (13) reported that lung parasites (metastrongylids) mainly degraded the condition and retarded the growth of the animals. The infestation of the lungs in swine is mostly associated with respiratory disturbances. As examples of this, *Ascaris suum* and *Metastrongylus* spp. are known to cause verminous bronchitis and pneumonia in pigs (13, 15). Metastrongylid infections often are complicated by secondary bacterial and viral infections and end fatally (8). Patra *et al.* (13) also stated that larvae of swine lungworms can carry the swine fever virus and some forms of stress can increase the virus pathogenicity.

Histopathological examination of organs or tissues facilitates a thorough and accurate diagnosis. Very often, histopathological examination allows the parasite involved, the area of pathological lesions, possible complications of bacterial or viral origin, and the outlook for treatment to be elucidated. Histopathological examination provides insight into interactions between pathogens and their impact on the host organism (17).

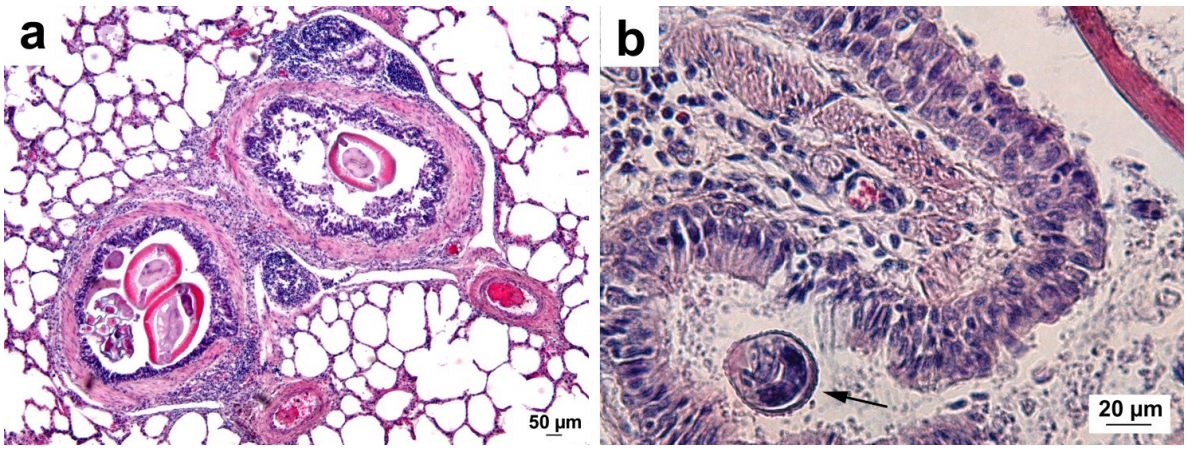

Fig. 1. Wild boar lungs with *Metastrongylus* spp. infection (HE): a – bronchial lumen with adult parasites; b – metastrongylid egg in bronchial lumen (arrow)

In connection with susceptibility to parasite infestation of swine and the utility of histopathology, the purpose of this study was to document the pathomorphological characteristics of helminth-infected internal organs of wild boars.

Material and Methods

The lungs and gastrointestinal tracts of 11 wild boars (*Sus scrofa scrofa* L.) were investigated from 2016 to 2018 in Bulgaria. The animals were selectively hunted complying with the requirements of Ordinance No 22/14.12.2005 on reducing the suffering of animals to a minimum at the time of slaughter or killing (promulgated in SG 22 of 14 Dec 2005 and transposing Directive 93/119/EC on the protection of animals at the time of slaughter or killing) and European Union Guidelines for the Accommodation and Care of Animals Used for Experimental and Other Scientific Purposes (2007/526/EC).

None of the animals had any lesions pathognomonic for acute viral or bacterial diseases. The sample materials were obtained during the carcasses' dissection and transported immediately to the laboratory for further examination. Firstly, a topographic sketch of superficial changes in the lungs was made. After that necropsies of the lungs were performed and the helminthological status of each lung was determined with a common method as described in a textbook (5). Parts of each altered area of the lungs were investigated in order to establish the helminth species which caused the particular lesion. Gastrointestinal tracts were also necropsied by a standard technique (5). The species identification of the helminths found was made on the basis of their morphometric characteristics after clearing some of the specimens in lactophenol.

Parts (0.5 × 2 × 2 cm) from the lesion-affected and helminthologically analysed portions of the lungs and gastrointestinal tracts were fixed in 10% buffered formalin, embedded in paraffin, cut into 5 to 6 µm thick sections and stained with haematoxylin and eosin (HE) for histological examination according to the routine histological technique.

Results

Lungs. Nematodes of *Metastrongylus* spp. were found in the lungs in 8 of the 11 animals. Helminths (infection intensity from 8 to 350, mean 164) were found in the middle and small bronchi, more often in the small ones. Macroscopic parasite lesions were located within the apical parts of the large lobes. They were irregular in form, pale greyish in colour, and compact in consistency, and nodules with a size of 5–15 mm could be palpated in them. When infection intensity was under 25, macroscopic lung lesions were not observed.

The histological investigations showed abnormalities in the lung parenchyma and interstitium. Sections of adult parasites and their eggs were the most frequent findings in the lumen of bronchi and bronchioles (Fig. 1). These findings were accompanied by hyperplasia of the bronchial epithelium and destruction and desquamation of epithelial cells from the bronchial mucosa. Remnants of desquamated cells, inflammatory cells (lymphocytes, neutrophils, eosinophils, and macrophages), and mucus were observed in the bronchial lumen (Fig. 2).

In the areas affected by parasites, moderate to severe inflammatory infiltration, thickening of the bronchial walls, and pronounced bronchiolitis and alveolitis were found. In some cases extravasation of erythrocytes into the bronchiolar lumen was observed. Parasites were not found in the alveoli. However, in many cases a thickening of the alveolar septa as a consequence of inflammatory infiltration with lymphocytes, polymorphonuclear cells, macrophages,

and Langhans giant cells were observed. In some cases in the affected lung interstitium parasite granulomas consisting of epithelioid cells and giant cells of Langhans type were noticed. They were surrounded by accumulations of mononuclear cells, neutrophils, and eosinophils (Fig. 3). Near to infected bronchi, areas with atelectasis due to alveolar collapse were seen. Focal activation and proliferation of lymphoid cells, as well as pulmonary fibrosis were also perceptible (Fig. 4).

Peribronchial and disseminated lymphoid hyperplasia was a frequent finding in the altered areas. In some cases, congestion of the alveolar septa was observed. Changes in the areas with atelectasis and development of compensatory alveolar emphysema were found in all infected lungs.

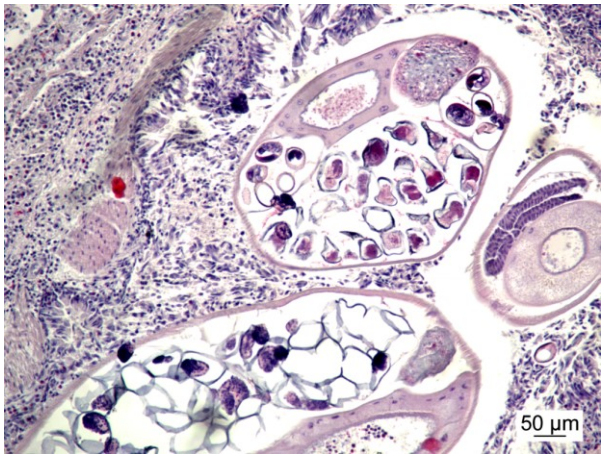

Fig. 2. Wild boar lungs with *Metastrongylus* spp. infection (HE). Inflammatory infiltrate by lymphocytes and neutrophils. Parasite forms, hyperplasia, destruction, and desquamation of the bronchial epithelium

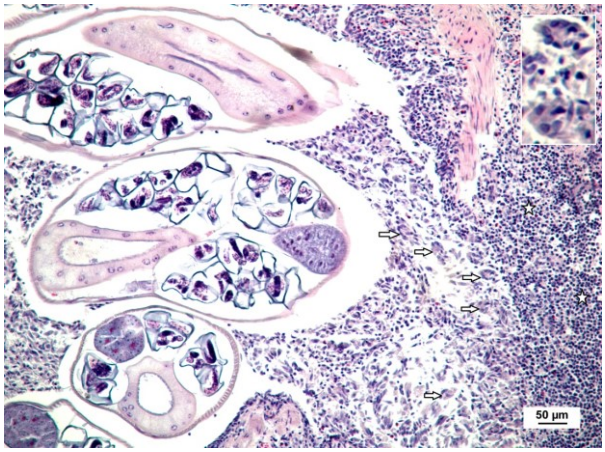

Fig. 3. Wild boar lungs with *Metastrongylus* spp. infection (HE). Granulomatous inflammation with presence of Langhans giant cells (block arrows; picture in the right upper corner)

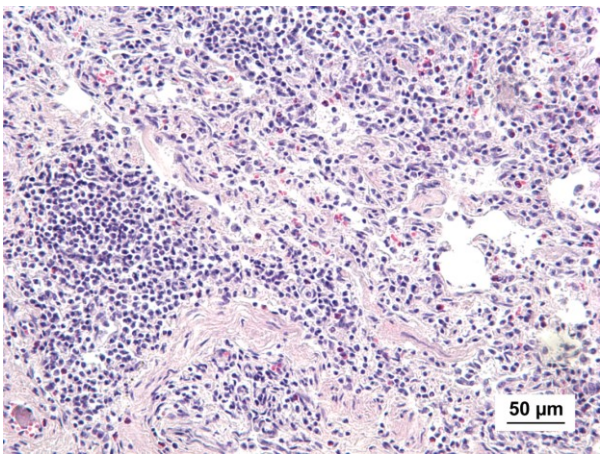

Fig. 4. Wild boar lungs with *Metastrongylus* spp. infection (HE). Interstitial inflammation, proliferation of lymphoid cells, and development of connective tissue replacing respiratory parenchyma

Stomach. Infection with *Ascarops strongylina* was found in three boars. The worms were located under a 2–3 mm mucus layer. Round pale-red lesions in the mucosa were also observed. The biggest of them were about 5 mm in size. Histopathological investigations of the changed stomach parts showed focal mucosal necrosis as the main lesion type. Clear demarcation of the mucosal and gland destruction was observed, formed by lymphocytes, neutrophils, and macrophages enclosing the periphery of the necrotic debris. This defect was lined by a thin fibrous capsule. The central portion of the necrosis is shown in Fig. 5.

Small intestines. Acanthocephalids of the *Macracanthorhynchus hirudinaceus* species were found in the small intestines of three animals. The parasites were strongly attached to the intestinal wall. These parts of the intestinal wall together with the attached helminths were exsected and put in saline solution. The worms had detached from the wall after 12 h. In an area around the parasite attachment extending to a 5–6 mm margin, the intestinal wall was twice as thick as normal. At the place of the parasite attachment, a small hole was observed, surrounded by an area of red tissue with a radius approximately the size of a lentil. The histological examination of the altered regions showed severe damage of the tissue with an intense inflammatory reaction. Perforation was observed in the place of the parasite attachment. It involved all layers of the intestinal wall apart from the serosa. Intestinal villi and their epithelium were destroyed in that site. The underlying mucosa and submucosa were also severely damaged (Fig. 6). Hyalinisation of fibrotic tissues was observed, developing around the proboscis of the acanthocephalan during its attachment and forming the gut wall defect. Peripherally, in the depth of the parasitic hole, a tissue reaction was found with a large amount of lymphocytes, polymorphonuclear leukocytes, and macrophages.

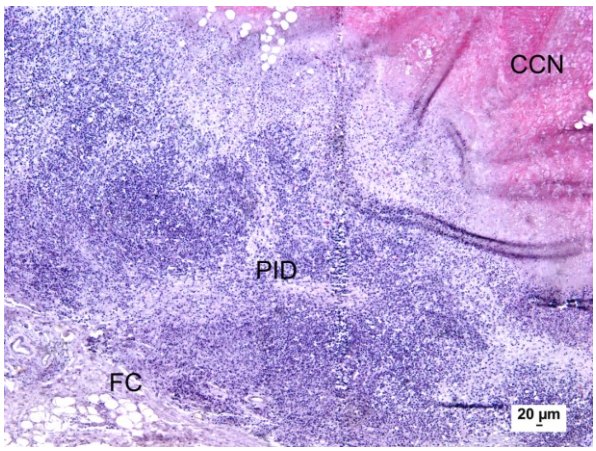

Fig. 5. Wild boar stomach with *Ascarops strongylina* infection (HE). Parallel cut of the stomach wall, part of the pathological defect. CCN – central caseous necrosis; PID – peripheral inflammatory demarcation of lymphocytes and neutrophils; FC – thin fibrous capsulation

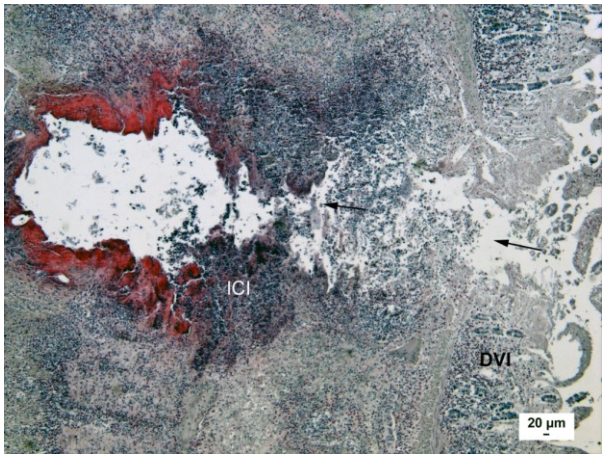

Fig. 6. Wild boar small intestine with *Macracanthorhynchus hirudinaceus* infection (HE). Perforative trans-tissue defect by the attachment of the parasite. Arrows – parasite hole; DVI – destruction of villi intestinales; ICI – inflammatory cell infiltration around the site of attachment

Discussion

The gross lung lesions established in the present study were located within the apical parts of the large lobes. Their location coincides with that of the parasite alterations reported by other authors who investigated domestic and wild pigs infected with metastrongylids: diaphragmatic lobes particularly in the caudal portion (6), mainly the lower parts of the main lobes (17), and caudodorsal borders of both diaphragmatic lobes (2). According to Marruchella *et al*. (8) both lungs parasitised with metastrongylids were diffusely firm. We established compact consistency only in limited, macroscopically altered lung parts. Focally dense consistency was also found by Sołtysiak *et al*. (17). It is possible that the difference between our observation and that of Marruchella *et al.* (8) is due to their study on the parasite infection having been complicated by an infection with type 2 porcine circovirus.

The lesions in the lungs of wild boars were irregular in form, pale greyish in colour, and compact in consistency, and nodules with sizes between that of a pea and a bean could be palpated in them. Comparing the macroscopic view of the changes that we found with that observed in previous studies of other animal species infected with lung worms (11, 12) we see that they are different. In goats, the gross lung lesions associated with *Muellerius capillaris* infections were mainly nodular, firm, and grey. Nodular lesions were also perceptible in the cases of *M. tenuispiculatus, Cystocaulus ocreatus, Neostrongylus linearis*, and *Protostrongylus brevispiculum* infections in the different ruminants. *P. rufescens*, *P. hobmaieri,* and *P. rupicaprae* produced large, diffuse dark red macroscopic lesions, commonly situated along the large bronchi (11). Brown-black nodes clearly differentiated from the surrounding tissue were the changes in red deer lungs infected with *Varestrongylus sagittatus*. More varied but also typical were lung lesions associated with the extrapulmonary protostrongylid *Elaphostrongylus cervi* (12). Acknowledging this diversity we could say that the macroscopic view of the pathomorphological lung changes in animals infected with lung worms depends both on the species of the parasite and the species of the host.

The main pathohistological findings in the present study could be summarised as follows: in the bronchi and bronchioles the presence of parasite forms, lymphocytes and neutrophils in their lumen, and desquamation of the bronchial and bronchiolar epithelium; in the alveoli alveolar emphysema; in the alveolar septa thickening and hyperaemia; in the interstitium alveolitis, giant cell reaction, and infiltration with monocytes and eosinophils; and presence of peribronchial and disseminated lymphoid hyperplasia. Such alterations to one degree or another were observed by other authors in studies on the pathogenesis of the metastrongylids in domestic and wild pigs (2, 6–8, 13, 17).

The microscopic abnormalities varied in type and severity both in the affected lung parts and the different animals. This is probably due to stage differences in the development of the parasitic infection when the studied lung lesions of the individual animals were formed. Validation for this statement is provided by the investigation by Mackenzie (7), according to whom the experimental metastrongylidosis in domestic pigs manifested different histopathology in the prepatent, early patent, and late patent stages of the invasion. For example, in our study, bronchitis and bronchiolitis were common findings for infected lungs that corresponded to the early stages of the parasitic infection and only in some cases did we detect fibrosis of pulmonary parenchyma, which is associated with a chronification of the infection.

Destruction of the mucosa and underlying glands by adult worms was noted in the stomachs of rabbits and guinea pigs experimentally infected with *A. strongylina* (1). An increase in thick mucus and catarrhal gastritis were the only changes observed by Sinha (16) and Karanja *et al*. (3) in domestic pigs infected with *A. strongylina*. Focal mucosal necrosis, which we found in the current study, was also observed by Karanja *et al*. (3) but in infections with other species of stomach nematodes: *Physocephalus sexulatus* and *Hyostrongylus rubidus*.

A severe destructive effect on intestinal villi, mucosa, and submucosa at the attachment site of *M. hirudinaceus* was also observed by Nelson and Nickol (10), Mowlavi *et al.* (9), and Sarkari *et al.* (14). According to Mowlavi *et al.* (9), in old lesions, the worms might give up their anchored sites the intestinal wall. They revealed an empty parasite hole which showed a chronic formation of fibrotic tissues. The present authors' observation of reparative replacement of necrotic tissues at the place of mechanical damage with newly formed fibrous tissue, which is gradually being hyalinised, also testifies to the chronification of the process.

In conclusion, the observed pathomorphological changes in the lungs and gastrointestinal tract of wild boars resulting from infection with *Metastrongylus* spp., *A. strongylina*, and *M. hirudinaceus* show a wide variety, especially those in the lungs. They vary from mild to severe, but none of them were lethal. The boars' living through infestation is most likely the result of co-evolution of the parasites and host, which allows the survival of both sides, as well as the result of their being spared complications from secondary viral, bacterial, or other infections.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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