

Original papers

Histopathological diagnosis in parasitic diseases

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ABSTRACT. Histopathological research is very important in diagnosing human and animal diseases. Detection of histopathological changes during certain parasitic invasions is particularly important for differential diagnosis and often confirms the presence of parasitic diseases. Such studies allow also to conclude on the primary cause of the disease.

Key words: histopathological research, parasites, tissue changes

Introduction

Histopathology represents a science on structure and function of tissues in ill individuals. Histopathological analysis plays an important role in the diagnosis of human and animal diseases of different etiologies such as infectious, neoplastic, parasitic, deficiency diseases and of intoxications. In many cases, parasitic diseases are not properly recognized. A cursory diagnosis often leads to prolonged or even ineffective treatment. Histopathological examination of organs or tissues facilitates a thorough and accurate diagnosis. Very often, histopathological examination allows to identify the parasite species involved, the area of pathological lesions, possible complications of bacterial or viral origin, and the outlook of treatment. Histopathological examination provides insight into interactions between pathogens and their impact on the host organism [1–3]. Detection of histopathological changes during certain parasitic invasions is particularly important for differential diagnosis and often confirms the presence of parasitic diseases.

This study was aimed at determining the role of histopathology in the diagnosis of animal parasitic diseases and revealing pathological changes occurring during invasions caused by different parasite taxa: the Protozoa, Trematoda, Cestoda, Nematoda, Acari and Insecta.

Materials and Methods

Tissue samples were collected from various animal species (turkey, chickens, reptiles, rabbits, pigs, wild boars, fish and dogs) suspected of carrying a parasitic invasion. The materials had been collected for many years and kept as demonstration specimens at classes of veterinary medicine at the Division of Parasitology. The materials obtained were fixed in 8% formalin. Histological tissue samples were stained with haematoxylin and eosin (H-E).

Results and Discussion

Most common histopathological changes caused by protozoans

The histopathological analysis of the large intestines and livers of turkeys infected revealed numerous oval or circular protozoan parasites with granular endoplasm, representing *Histomonas meleagridis* (Fig. 1). Histopathological examination of the large intestine and liver of reptiles revealed the presence of *Entamoeba invadens*. These

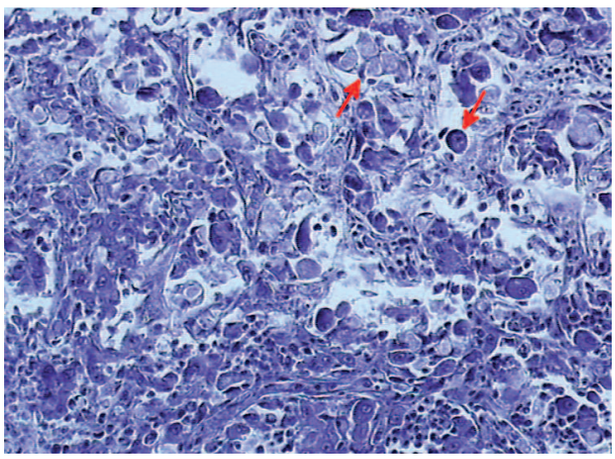

Fig. 1. *Histomonas meleagridis* – the histological structures of liver are damaged, numerous oval or circular protozoan parasites with granular endoplasm are surrounded by the inflammatory cells. H-E, mag. 100x.

protozoan parasites are oval in shape, their nuclei and karyosomes being well-stained. According to Brener et al. [4], the presence of a parasitic disease in birds can in many cases be confirmed despite the absence of clinical signs. The study showed cellular and topographic changes of the liver and caecum in turkeys infected with *H. meleagridis*, thus confirming the pattern of the hepatic and caecal infections by *H. meleagridis* in birds.

The cross-sections of the intestinal wall of pigs infected with *Balantidium coli* revealed round or oval protozoan parasites with bean-shaped macronuclei. The protozoans surrounded the inflammatory infiltrate composed of lymphocytes, histiocytes, and eosinophiles (Fig. 2). In coccidiosis-affected rabbits, the schizogony and gametogony damaged the biliary epithelium and

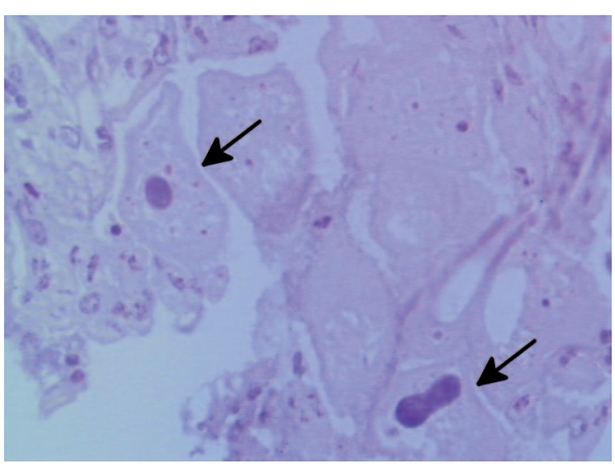

Fig. 2. The large intestine of pigs infected with *Balantidium coli* revealed round or oval protozoan parasites with bean-shaped macronuclei. H-E, mag.100x.

produced clinical symptoms. The coccidia schizogony and gamogony in some rabbit species occur in the small or large intestine producing histologically detectable inflammatory changes. Thin gamonts of *Eimeria necatrix*, *E. acervulina*, *E. precox*, *E. mivati*, *E. maxima*, *E. brunetti* and *E. tenella* were visible within the epithelial cells of the small and large intestinal mucosa of chickens. The cross-sections of the intermediate host's skeletal muscles revealed many zoites (*Sarcocystis* spp.) within sarcocysts (the Miescher cysts) [5,6].

Histopathological changes caused by flatworms

Liver fluke disease of sheep and cattle, and occasionally other species, most commonly is due to the juvenile *Fasciola hepatica* fluke injures the hepatic parenchyma when moving to the bile ducts. Migration of immature flukes through the liver produces hemorrhagic tracts of necrotic liver parenchyma. These tracts are grossy visible and in acute infestation, are dark red, but with time become paler than the surrounding parenchyma. Repair process is often by fibrosis. The *Schistosoma*

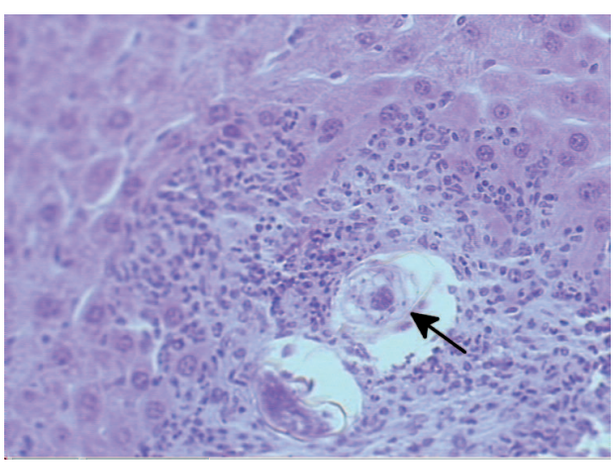

Fig. 3. Eggs of *Schistosoma mansoni* surrounded by inflammatory cells. H-E, mag.100x.

haematobium fluke eggs use their spine to penetrate the wall of the urinary bladder and enter the external environment with the host's urine. The *Schistosoma mansoni* fluke eggs break through the wall of the colon into the gastrointestinal tract and in doing so damage the lamina and mucosa of the large intestine. The masses of eggs become surrounded by inflamed areas infiltrated by leucocytes, particularly eosinophiles, in internal organs such as the liver (Fig. 3).

The larval form of *Taenia pisiformis* burrows tunnels through the hepatic parenchyma of rabbits,

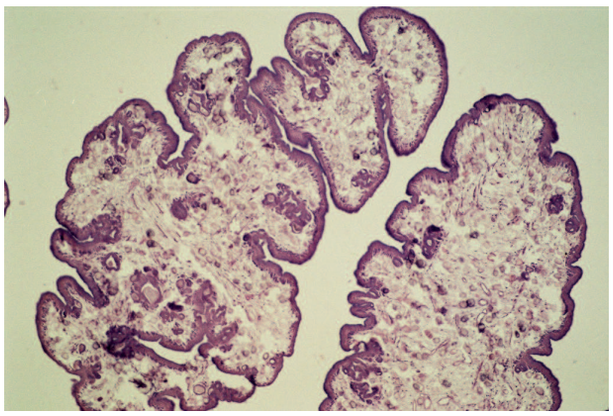

Fig. 4. The *Tetrathyridium* larvae covert by tegument - inside are present the lime glands. H-E, mag.100x.

damaging their liver parenchyma and inducing the formation of a peripheral inflammatory infiltrate composed of eosinophiles and giant cells. In chronic cases, the damaged liver parenchyma produces fibrotic scars. The second larval form of *Mesocestoides* spp. is the *tetrathyridium* found in the avian and reptilian body cavity, and occasionally found in the abdominal cavity of dogs (Fig. 4). The larvae of *Echinococcus multilocularis s. alveolaris* are often located in the liver, lungs or brain. The larval forms are surrounded by inflammatory infiltration with numerous eosinophiles, necrosis and calcification [6]. The diagnosis is confirmed by histological detection of *Echinococcus* larval hooks.

Histopathological changes caused by roundworms

The Anisakidae nematodes living in marine fish and mammals from the northern and southern hemispheres are parasites of veterinary, medical, and economic importance. Adults of most of them dwell in the alimentary tract of marine vertebrate hosts (cetaceans and pinnipeds). The Delphinidae are the main final host of *Anisakis* spp. Adults of *Pseudoterranova decipiens* (sensu lato) and *Contracaecum osculatum* (sensu lato) are found mainly in the Otariidae and Phocidae [7,8]. Histological examination showed L4 and adult parasites attached to the gastric wall. The anterior parts of anisakids attached to the gastric mucosa and submucosa were ruptured and were also associated with ulceration. A mucosa surrounding the anisakids revealed the presence of more or less confluent focal necrotic areas. Most small petechial haemorrhages were located in the gastric wall mucosa and were surrounded by inflammatory mononuclear cells such as lymphocytes, histiocytes, eosinophiles and fibroblasts. The necrotic areas showed, located in their centres, single or multiple parasitic elements of irregular shape, with a thick segmented cuticle covering the dorsal and ventral musculature; the lateral, dorsal and ventral chords were observed. Inside the pseudocoel, the elements resembling gastroenteric-like structures and lateral chords of the parasite were detected. The worms provoked a surrounding granulomatous reaction, containing a central core of necrotic and cellular debris and a large number of eosinophiles. The lesions exhibited an inflammatory response of the lamina mucosa and submucosa, but did not reach the gastric muscularis. The gastric glands near the parasite attachment were damaged. The Anisakidae larvae penetrated deep into the stomach wall and induced haemorrhages and eosinophile infiltrations causing atrophy and sometimes formation of small cysts, leading to the formation of tissue scars with multiple fibroblasts. Necrotic foci were sometimes calcified.

The lungs of wild boars were damaged by the *Metastrongylus* spp. Houszka [9] demonstrated the prevalence occurring in wild boars in Poland an open environment oscillates between 50–89% and up to 100%. The death of 14 young boars in the early spring of 2000 is given as an example where extensive metastrongylosis with consecutive pneumonia was diagnosed.

The wild boar lungs examined were focally congested, dense in consistence; the altered pulmonary parenchyma in a water test was airless. Parasitic changes in the lungs were present in all the wild boars examined. Dark red lobuli were surrounded by centrilobular emphysema light pink in colour. The pulmonary pleura in place in the centrilobular emphysema was slightly elevated. The changes affected mainly the lower parts of the main lobes of both lungs. The enlarged light bronchioles and bronchi revealed the presence of numerous nematodes in the bronchiolitis mucus. Abundant nematodes in the bronchioli (over 20 nematodes) cause the extending the cross-section of brioncholi. Abundant nematodes in the bronchioli (over 20 nematodes) cause the dilatation of the bronchioli light. The presence of adult nematodes in the bronchial tree, their movements and their excreta as well as the antigens secreted cause inflammation of the bronchial tree, and then inflammation in the tissue and alveoli causing bronchopneumonia. Larvae migrating through the bronchial alveoli also damage alveoli the walls and cause lung tissue

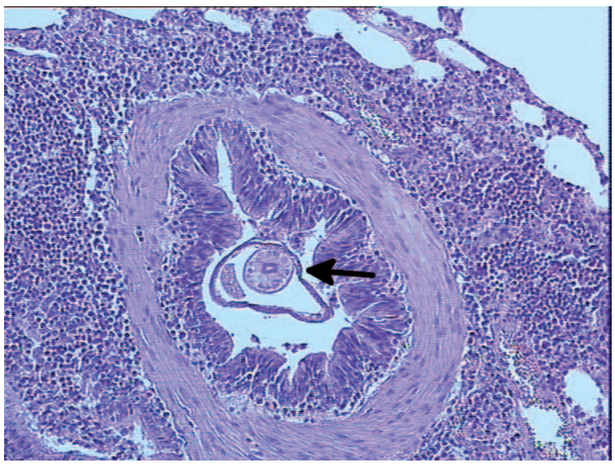

Fig. 5. Cross-sections of the bronchus surrounded by inflammatory process with *Metastrongylus* spp. parasite inside bronchus. H-E, mag.100x.

inflammation. The larval nematode migration in the pulmonary parenchyma to the bronchi and bronchioles caused changes and trauma leading to the development of inflammation expressed by infiltration of inflammatory cells with numerous eosinophiles (Fig. 5). The mechanical damage healed through scarring, i.e., the formation of the connective scar tissue involved in the repair process leading to extensive lung fibrosis. In the lung parenchyma was also reported small necrotic foci, that have been calcified. Mobility of nematodes and antigens secreted, their excreta, and the presence of eggs resulted in chronic bronchitis and bronchiolitis with eosinophilic cells. Migrations of larval *Metastrongylus* nematodes in the lung parenchyma caused catarrhal pneumonia, the secondary purulent infection being caused by staphylococci (*Staphylococcus* spp.).

Respiratory tract infections are caused by parasites, bacteria or viruses, and mixed respiratory tract infections are very common. The interaction of pathogens exacerbates the disease, whereby pathological changes are more complicated [10].

Numerous authors confirmed the usefulness of histopathological examination in evaluation of the disease process. Marruchella et al. [11] demonstrated that a concurrent porcine circovirus type 2 may trigger metastrongylosis, which may subsequently result in severe, and sometimes fatal, pulmonary disease. Histopathological analysis helped to fully determine the causes of the disease in the animals affected.

Histopathological changes caused by arthropods

In dogs, burrowing mites (*Sarcoptes scabiei v. canis*) parasitize in the deep layers of the epidermis (stratum granulosum, s. spinosum). The changes are located on the head, rump and at the base of the tail. At the beginning of the invasion, the lesions occur as local, limited skin exfoliation which next creates scaly raids. As the disease progresses, the skin is reddened and thickened, and corneous epidermis forms papule.

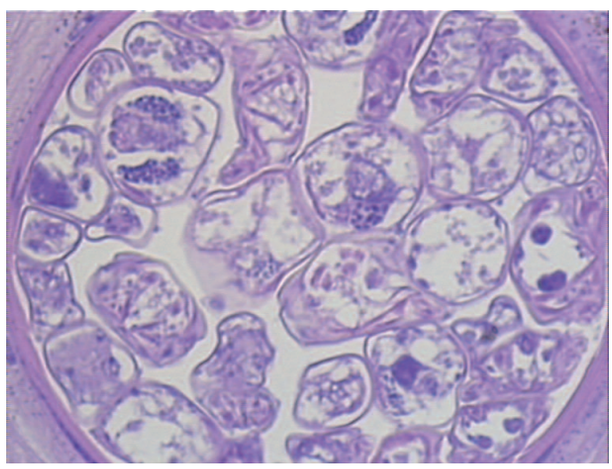

Fig. 6. Cross-sections of the *Demodex canis* in the hair follicle. H-E, mag.100x.

Demodex canis occupies the hair follicles and sebaceous glands and causes generation of small nodules with thinned hair, and excessive skin desquamatio. Initially, the changes appear on the head, then small lesions form larger, clearly demarcated, red scalp patches extending to the neck, forelegs and the rest of the body. The disease is chronic (Fig. 6).

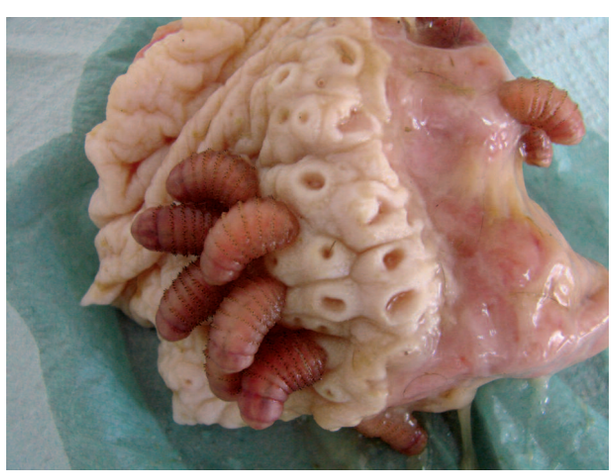

Fig. 7a. The III stage of *Gasterophilus intestinalis* larve present in horse's non glandular stomach

The presence of *Oestrus ovis* larvae causes inflammation of the mucous membrane of the nasal cavity and sinus. Occasionally, the larvae may penetrate the skull bones and enter the cerebral cavity; clinical signs are similar to those caused by *Taenia multiceps* called the false gid. Changes in the mucous membrane of frontal sinus include catarrh, inflammatory cells infiltration and squamous metaplasia. The points of attachment of L2 and L3 larvae of *Gasterophilus intestinalis* in the glandular and non-glandular stomach of horses are marked by wastages on both the corneous layer and the squamous mucosa, accompanied by its focal hyperkeratosis (Fig. 7a). The necrosis focus showed bacterial colonies and infiltration of eosinophiles and neutrophiles. The bottom of the wastage in the proper epidermal layer of the mucous membrane featured diffuse infiltrates of inflammatory cells (Fig. 7b).

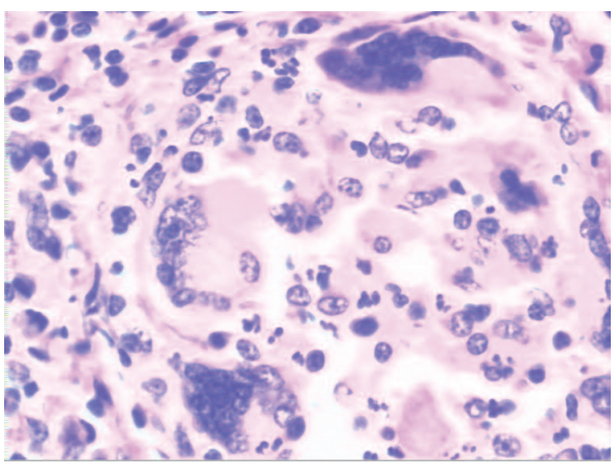

Fig. 7b. Diffuse infiltrates of inflammatory cells especially giant cells during *Gasterophilus intestinalis* larvae infestation. H-E, mag. 400x.

Conclusions

Histopathological examination is useful for a number of reasons: a/ to detect parasites; b/ to reveal the area of tissue damage caused by migrating larval forms and mature parasites; c/ to apply appropriate treatment; d/ to explain why certain treatments are not effective during a parasite invasion (parasites damage parenchymal organs causing permanent organ dysfunction and inducing formation of fibrotic scars). Histopathological examination provides insights into interactions between pathogens and their impacts on the host organism, particularly in poly-etiological infections.

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