PATHOMORPHOLOGICAL CHANGES IN ROE DEER LUNGS DURING ONTOGENESIS OF VARESTRONGYLUS CAPREOLI (STROH ET SCHMID, 1938) DOUGHERTY, 1945

R. ŠVARC and A. PAJERSKÝ

Institute of Helminthology, Slovak Academy of Sciences, Košice, Czechoslovakia

Abstract. The specification of pathomorphological changes occurring during Varestrongylus capreoli ontogenesis was studied on the basis of different forms of inflammation. The character, course and extent of inflammatory reactions were contingent on the maturity, localization and number of worms. The allergic inflammation accompanying the development of juvenile specimens inside the alveoli was followed by the regeneration of lung tissues providing suitable conditions for further development of the parasite. A prolonged edema occurring during the differentiation of juvenile parasites localized in the bronchioles caused a disposition of the rough bronchial wall to its milky necrotic disintegration producing a negative effect on further development of the helminth. The pathomorphological effect of mature productive parasites was characterized by focal changes — hacking nodes. Activated epithelial cells of alveoli, phagocytizing disintegrating eggs and not developing late-stage larvae together with eosinophile granulocytes participated in the restoration of the host lung functional ability while tolerating the presence of the parasite. A difficult excretion of sex products from the lungs stimulated the development of proliferative forms of inflammation reducing the number of Varestrongylus specimens.

The lungs of roe deer may be infected by several nematode species together, which makes it very difficult to determine the pathomorphological changes induced by individual species. The helminth fauna of ruminant hooved game has been dealt with in numerous papers (Bayer 1938, Boev 1957, Erhardová 1957, Kotrly 1958, Boev et al. 1962, Erhardová-Kotrly and Ryšavý 1967, Tománek 1967, Mitoč 1969, Zilinčár 1984). According to Kotrly and Krul (1960), the most common parasites infecting roe deer lungs in Czechoslovakia are Caproncclus capreoli and Dictyocaulus viviparous. The occurrence of Muellerius capillaris detected in one case seems to be related with the locality at which sheep were pastured previously. None of the papers available to us reported about pathomorphological changes caused by these worms in the lungs of roe deer. In some papers (Marsh 1938, Veselodov and Boev 1948, Erhardová et al. 1953, Sogovsky 1956, Kotrly and Krul 1958, Petrosyan 1963, Forrester and Senger 1963, Krul and Schanzl 1967) the lung lesions are ascribed to a group of helminths without their more exact identification. In our materials, which had been collected for several years, we have separated lungs infected by a single species specific for the roe deer, which was named by Boev (1975) Varestrongylus capreoli (Stroh et Schmid, 1938) Dougherty, 1945. The original name Caproncclus capreoli (Stroh et Schmid, 1938) Schulz et Kadenzsi, 1948, which was formerly accepted by this author, is now considered by him to be a synonym to which he places also Protosstrongylus capreoli Stroh et Schmid, 1938. In the present paper, the newest name of this lung nematode is used and the disease caused by it is termed varestrongylosis of hoofed game.
MATERIALS AND METHODS

The lungs were examined both macroscopically and microscopically. The worms were found in smears from sections, in preserved preparations, and in histological sections made from pathologically changed lungs. Morphological criteria of adult worms, tail ends of larval-stage larvae, and the specific process of egg passage were used for the identification of *Varestrongylus coreyi*. Among the 48 lungs examined, there were 20 lungs infected only by this nematode species. The maturity of worms was estimated on the basis of their size, organ structure, and sexual activity. Lung excisions were taken from all forms of macroscopical changes. Histological preparations were prepared using a common laboratory technique of paraffin sections stained by hematoxylin-eosin.

RESULTS

Lung changes stimulated by juvenile worms

Lung affections appearing after the transfer of postinfective larvae from the blood into lungs are the first of the pathomorphological symptoms of spontaneous varestrongylosis which will be evaluated in the present study. In a macroscopic picture of the surface of deer lungs they appear like irregular, radial brown-red areas, the extent of which depends on the number of worms. After their fusion their size is up to that of a pea, but they do not protrude more markedly. If they are surrounded by hyperemic lung tissues, they get the more diffused character of infiltration. In case of a more advanced hypoplasia or in lungs poorly supplied with blood or damaged by perforation it is more difficult to see and classify them. In the lung parenchyma their darker color contrasts with the surrounding tissue. The presence of a serious exudate indicates that an exudative inflammatory process is in progress inside them.

The exudative inflammation in the nodule affections was confirmed by histological studies. The emigrated eosinophilic granulocytes dominated in the cellulization and therefore the inflammatory process was classified as an allergic one. Individual stages of the allergic inflammation correlated with the progressing differentiation process of juvenile varestrongylids, whereas their different localization in the alveoli or bronchi influenced the course of the allergic inflammation. It resulted in lung changes differing both macro- and microscopically.

Development of lung changes induced by juvenile worms localized in alveoli

If the juvenile varestrongylids in the inflammation areas develop in the alveoli, the allergic inflammation persists in its manifest stage during the whole structural development of the worms (PI. 1, Fig. 1). A dark brown running excentrically along the worm body cavity shows the worm differentiation. This morphological functional reconstruction takes place shortly after the transition of postinfective larvae from the blood into lungs. No varestrongylids in regenerating alveoli (PI. 1, Fig. 3) were found at the final stage of allergic inflammation. The good staining ability of cuticular layers, gradually lighter color of the worm cell cytoplasm, and finely granular homogeneous contents of the uterus with eggs at the early stage of formation indicated that the varestrongylids, particularly their females, had terminated their juvenile stage and had reached sexual maturity. During the study of numerous series of histological sections, a male spirally enveloping a female was found. This physiological character of varestrongylids, which is specific also for other protosopyridyl species, shows the biological association with the production of progeny.

The course of the allergic inflammation, as observed microscopically, is reflected also in the macroscopic picture of the lung changes. The original inflammatory areas get a map-like appearance during the differentiation process of varestrongylids and gradually face out or disappear from the pathomorphological picture. If the lung surface is examined immediately after the animal has been killed, the inflammatory areas appear like light spots, but they escape attention in the parenchyma. The examination performed by compressing the tissue, which showed at least indications of these slight macroscopic changes, confirmed that they originated from the varestrongylids. Just in these changes we detected histologically the sexual difference between the worms influencing the further development of the changes caused by them. This will be discussed in another chapter.

Our studies showed that the allergic inflammation accompanying the differentiation process in juvenile varestrongylids localized in alveoli terminated by a functional regeneration of lung tissues which created suitable conditions for a further development of the parasite. Although the changes observed macro- and microscopically were pathognomically convincing and from the viewpoint of worm pathogenicity negligible, their diagnostic importance consisted in that they showed the sites at which the preimaginal stages of parasites infected the lungs.

Development of lung changes caused by juvenile varestrongylids localized in bronchioli

The differentiation of juvenile worms parasitizing the bronchioli makes more intensive the exudative component of the allergic inflammation in the original areal affections, which to a great extent influences its further course. The inflammation areas become turbid due to the edematous pleura, but they are not markedly limited. After fusion these changes become adequately more extensive and marked. The inflammatory edema creates in them a disposition to necrotic disintegration of lung tissues. In the macroscopic picture of deer lung the necrosis is visible in form of dense planary military configurations resembling a spread semolina. This regressive character of lung changes transforms the original inflammatory areas to nodular ones. As it is visible in the section, they are deeply embedded into the parenchyma from which rough walls of bronchioli markedly protrude. The sexual dimorphism of parasites is already morphologically apparent and the formation process continues to persist again. It should be noted that the miliary necrotic changes associated with the infection were not adequate to the number of compression examinations. However, the miliary necrosis was the most typical and dominant type of pathomorphological changes in the macroscopical picture.

Histological examinations of the excisions from miliary necrotic changes confirmed that the edemas persisting in the allergic inflammatory process is stimulated by juvenile varestrongylids developing in the bronchioli (PI. 1, Fig. 4). A disintegration of cell nuclei and alveoli were observed in the rough wall of bronchioli, which indicates the beginning of necrosis (PI. 1, Fig. 5). A developed colliquation necrosis of the wall of bronchioli is shown in PI. 1, Fig. 6. At the same time, the inflammatory process affected negatively the development of parasites in the neighbouring alveoli. The parasites in eosinophilic granulomas became swollen and gradually disintegrated (PI. 2, Fig. 7). The necrotic parts of lung tissues and worms were phagocytized by cells forming large multinucleus symplings. These syncytia most probably originated from blood monocytes, because the activation of fixed cells of mesenchymal origin to inflammatory process was not very marked or was completely lacking. The empty places remaining after the necrotic disintegration were gradually
filled with cells of hyperplastic peribronchial and perivascular lymphatic tissues which formed diffuse or more often avold configurations. These lymphohemopoietic changes, together with the necrobiosis of bronchial walls, characterized the histo-pat-hological picture pathognomonic for developing varestraeglyids in bronchioli.

Lung changes induced by sexually productive worms

Sexually productive worms introduce into the process of changes a new significant pathogenic agent — eggs and the larvae which hatch from them. Their development will be described in relation with the lung changes which arose during the development of juvenile parasites into adults inside the alveoli and then inside the bronchioli. The pathomorphological changes which developed at the places where both male and female worms parasitized and which appeared in macroscopical examination as pale spots are termed “hatching nodes” in helminthology. They were localized in the most aeratal lung lobes where they predominated in form of irregularly rectangular or square foci 1–2 cm². Their martry appearance was caused by alternating atelectatic and emphysematous alveoli. Fragments of sexually productive worms, eggs at different stages of cleavage, and larvae fetched from it described this fact was revealed during microscopical observations of smears of wedge-shaped foci in the lung parenchyma. The internal structure of developing eggs is morphologically so conspicuos that, together with the characteristic tail end of larvae, it is considered to be suitable for the diagnostics of varestraeglyids (Pl. II, Fig. 8).

Histopathological examinations of the hatching nodes showed that the change in the number of inflammatory areas stimulated by developing parasites in alveoli, revealed the poor reactivity of host lung tissues caused by the undisturbed release of sexual products from lungs. If they accumulated, then atelectatic changes occurred in the hatching nodes. They infiltrated lymphocytic cells or resulted in the infiltration. Such changes, however, were not very frequent in the pathomorphological picture of the deer lungs.

The study of the development of macroscopical millary necrotic changes induced by the maturation of preimaginal parasite stages in bronchioli showed that they got a greater number of atelectatic and emphysematous changes when involved. Lobular structures formed on the surface of disintegrating lung lobes. They measured several centimetres and slightly promiment. The millary necrotic process gradually disappeared from them and they were replaced by minute homogeneous nodes grey in section. The fibrous pleura of focal changes became uniformly grey and demarcated. There occurred sexually mature worms and we therefore termed them, like the previous focal changes, hatching nodes. We consider these pathomorphological changes to be more characteristic of varestraeglyids in the deer lungs.

The histological picture of hatching nodes showed marked phagocytic reactions eliminating the unfertilized disintegrating eggs and immature, dark, granulated larve (Pl. I, Fig. 9). Eosinophilic granulocytes dominated in the emigrated blood during the processes, whereas the epithelial cells of alveoli capable of phagocytosis resembled the swelling of nuclei and enlargement of the glandular tissue cytoplasm at the transformation stage (Pl. II, Fig. 10). The histological picture showed also the rough wall of bronchioli and lymphaphemopoietic changes which arose during the differentiation of juvenile worms in bronchioli. The parasites were localized between alveoli and respiratory bronchioli (Pl. II, Fig. 11).

The focal changes in the distal parts of lungs occurred in the apexes of the lung lobes or along their margins as bands of various widths. The increasing emphysematous changes produced light spots in their initially grey colouring, but the dominating character of millary necrotic changes remained preserved in macroscopical and microscopical pictures. Even if such lung affections had often been encountered in the deer lungs, their parasitic origin had never been demonstrated.

Microscopical examinations of the focal changes situated in the apical parts of diaphragmatic lung lobes revealed that the necrobiosis in them persisted at the same stage of development (Pl. II, Fig. 12). This is explained by the fact that the examined lung excisions did not allow us to evaluate it at its early stage of transformation, not even at its final stage. We have therefore decided to evaluate these lung changes, together with those which could not be etiologically identified as varestraeglyids, on the basis of a complete series of histological sections. The results of these studies will be published in another paper.

DISCUSSION

The authors cited in the introduction, Kotrly (1958) considers Coproocaulus cupreus and P. troglodytes troglodytes to be a specific parasite of deer, as it was confirmed also by our studies in the catchment Tominek (1967), who recorded a 78 % infestation of deer lungs with this nematode.

The pathomorphological description of varestraeglyids, as a nosological part of protostomycylosis of free-living ruminants, has not been found in the available literature. This disease is known under the general term “lung worm disease” and due to the mentioned changes it makes a part of the pathomorphological picture of infected deer lungs.

While examining the pathological changes caused by lung worms Krul and Kotrly (1959) observed a hypertrophy of muscle layer in bronchial walls and presence of giant cells around the tissues disrupted by the inflammation and remains of parasites. The hatching nodes in deer lungs were described by Krul and Schannel (1967) as lobular emphysematous foci with nodes forming glittering, compact, beak-like masses after fusion. They consider the depletion of the peribronchial lymphoedemic tissues to be identical with the changes described by Jelinek (1956) as lymphadenopathy nodosa pulmonum. They conform to the lung changes described by us when the lung lobes and various regions induced by the differentiation of juvenile varestraeglyids in bronchioli was described.

A very variable pathomorphological picture was reported by Petrovyan (1963) in lungs of elk (Alces alces). The author found numerous solid nodes of a yellow-grey colour and of a size of pea or walnut under the pleura and in the parenchyma of the infected lungs. They differed from the surrounding lung parenchyma, slightly promiment and gave the lungs a rough appearance. Microscopically they resembled the ovine cystocaulous nodes, but they differed from them in the absence of typical cysts. The histological picture showed a chronic inflammatory process of lobular character in which dominated the giant cells phagocytizing the eggs and larvae. The author characterized the inflammatory process as the giant-cell pneumonia.

The above facts indicate that Varestraeglyus cupreus, even in case of a mixed infection with other nematode species, causes specific pathomorphological changes suggesting its presence in the deer lungs.

The pathomorphological studies, in which the development of lung changes was evaluated in the ontogenesis of Varestraeglyus cupreus, showed also some characters of the latent cycle of this nematode. The presence of juvenile parasites in the lungs of deer killed in areas where the disease can be regarded as a latent infection or it may be admitted that the host was infected even in this season. The deer often feed on dry grass.
and low shrubs, at which the hibernating intermediate hosts, as snails, may occur in the biotopes covered by snow. The dynamic course of different forms of inflammatory reactions at the time of infection with both juvenile and adult varestrongylids supports this assumption.

ПАТОМОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ЛЕГКИХ КОСУЛИ В ТЕЧЕНИЕ ОПОТЕНБЕЗА VARESTRONGYLUS CAPEROLI (STROH ET SCHMID, 1938) DOUGHERTY, 1945

П. Шварц и А. Паереки

Резюме. Испытана специфичность патоморфологических изменений, встречающихся в течении опотенебеза Varestrongylus capreoli на основе разных форм воспаления. Характер, течение и объем воспалительных реакций находятся в связи с повреждением, деструкцией и количеством червей. После аллергического воспаления, сопровождающего развитие кожно-органных червей в альвеолах, происходила ретроградация ткани легких, обусловливая убыль легких. Дальнейшее развитие патологии происходило на фоне хронического воспаления и некротического процесса, что способствовало отграничению очагов и дальнейшему развитию гемаглобиномы. Патоморфологическое влияние половых продуктов паразита было характеризовано местными изменениями — "catching nodes". Активированное эндотелиальные клетки альвеол, фагоцитирующие распадающиеся ядра и нервные включения первой стадии одного с эозинофильными гранулоцитами, участвовали в восстановлении функциональной способности легких хозяина, перевода присутствие паразита. Усиленное явление половых продуктов из легких стимулировало развитие пролиферативных форм воспаления, повышая количество паразитов.

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B. Š., Helminthologický ústav SAV, Dukelských hřištů 3, 040 01 Košice, ČSFR
Fig. 1. Juvenile worm in allergic inflammation (× 80).
Fig. 2. Mature worm in regenerated alveoli (× 80).
Fig. 3. Females in wall of alveoli pressed by emphysema (× 80).
Fig. 4. Varestrongylus in bronchiol (× 80).
Fig. 5. Disintegration of cell nuclei accompanied by malaria of bronchiol structures (× 180).
Fig. 6. Colligation necrosis of bronchiol wall (× 80). All figures stained with haematoxylin-cosin.

Fig. 1. Disintegration of juvenile varestrongylids (× 80).
Fig. 2. Eggs at various stages of cleavage (× 180).
Fig. 3. Phagocytic reaction in hatching nodules (× 180).
Fig. 4. Alveolar structure imitating glandular tissues (× 180).
Fig. 5. Sexually productive worm in hatching node (× 80).
Fig. 6. Miliary necrosis of lung tissues (× 40). All figures stained with haematoxylin-cosin.