sciendo

J Vet Res 63, 549-554, 2019
DOI:10.2478/jvetres-2019-0068

First description of histopathological lesions associated with a fatal infection of moose (*Alces alces*) with the liver fluke *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932

Katarzyna Filip-Hutsch[1], Tomasz Hutsch[2], Szymon Kolasa[3], Aleksander W. Demiaszkiewicz[1]

[1]W. Stefański Institute of Parasitology PAN, 00-818 Warsaw, Poland
[2]Department of Experimental Physiology and Pathophysiology, Laboratory of the Centre for Preclinical Research, Medical University of Warsaw, 02-106 Warsaw, Poland
[3]Polesie National Park, 22-234 Urszulin, Poland
katarzyna.filip@twarda.pan.pl

Received: March 28, 2019 *Accepted: October 30, 2019*

Abstract

Introduction: *Parafasciolopsis fasciolaemorpha* is a liver fluke typically parasitising moose in Central and Eastern Europe. The aim of our studies was to describe a case of fatal moose parafasciolopsosis, with special emphasis on the histopathological changes caused in the liver tissue by around 10,000 flukes. **Material and Methods:** A male moose, found dead in Polesie National Park, eastern Poland, was subjected to parasitological necropsy. Macroscopic and histopathological examination of the liver was performed. **Results:** Over 10,000 flukes identified as *P. fasciolaemorpha* were isolated from the liver parenchyma. Histopathological examination of the liver revealed the presence of multiple cavities, which were filled with flukes and cellular detritus and encysted with a layered capsule of connective tissue. Extensive liver fibrosis with signs of incomplete septal cirrhosis was also observed. **Conclusion:** Parafasciolopsosis with accompanying diarrhoea was the most probable reason for the moose's death. However, it is possible that most moose are able to survive extremely intensive *P. fasciolaemorpha* infection by formation of extensive fibrosis, which isolates flukes from the liver parenchyma and therefore retards the failure of the organ. To the best of our knowledge, this is the first histopathological description of changes in the liver of a moose infected with *P. fasciolaemorpha*.

Keywords: moose, *Parafasciolopsis fasciolaemorpha*, histopathology, liver fibrosis.

Introduction

Parafasciolopsis fasciolaemorpha Ejsmont, 1932 is a parasite of moose which resides in the bile ducts of the liver (1, 4). The life cycle of this fluke is typical of the family *Fasciolidae*, with the great ramshorn water snail (*Planorbarius corneus*) as its only intermediate host (10).

The fluke is commonly observed in moose in Central and Eastern Europe, which is home to a large population of great ramshorn snails (10). Parafasciolopsosis has been observed in 70% to 100% of examined moose in Poland. The infection typically proceeds with extremely high intensity, ranging from a few thousand to many thousands of trematodes in a single animal (2, 4, 18). The most intensive infection, recorded by Kazlauskas and Shlejjkus (7), was almost 118,000 flukes in a single moose. Parafasciolopsosis is therefore associated with the development of serious gross lesions in the liver tissue. Diarrhoea and a general worsening of condition occur over the course of the disease, leading to emaciation and the death of the host (1, 2, 3).

Although histopathological changes caused in the liver by other flukes from the family *Fasciolidae* are widely described (5, 11, 13), no data exist regarding *P. fasciolaemorpha*. As the fluke is also dangerous to other cervids and domestic ruminants (3), it is clearly

of great importance to evaluate the unique pathological changes resulting from *P. fasciolaemorpha* infection. The aim of this article is to describe a fatal case of moose parafasciolopsosis, with special emphasis on the histopathological changes caused by extremely advanced infection in liver tissue.

Material and Methods

Necropsy. A four-year-old male moose was found dead in February 2018 in Polesie National Park, eastern Poland (51°27′46.44″N, 23°10′47.17″E). Immediately after the discovery, the animal was subjected to field necropsy according to King *et al.* (8). Numerous loose faeces in the surroundings indicated that the moose suffered from diarrhoea. The animal also showed signs of emaciation. On the basis of organ assessment, only the liver and a faecal sample from the rectum were collected for further examination. The liver was subjected to macroscopic examination, palpation, and evaluation of extrahepatic bile ducts. Cross-sections of the liver parenchyma were taken, cutting it perpendicularly to the bile ducts.

Isolation and identification of trematodes and their eggs. After examination, the liver was cut into small pieces. Each slice was compressed so that flukes exited the bile ducts and was then rinsed with water. Mature flukes and eggs were then isolated from the decanted liver sediment as protocolled by Dróżdż (2). Trematodes were identified by morphometrical features (4, 18) and counted under a stereoscopic microscope (PZO, Poland) at 10× magnification.

Examination of moose faeces. A total of three grams of faecal sample were examined by the decantation method according to Taylor *et al.* (17). Eggs were identified to the species level by their morphometrical features (4).

Histological preparation and examination. Histological assessment was performed on tissues harvested from the liver. Tissue sections were fixed in 10% buffered formalin, dehydrated in graded ethanol and xylene baths, and embedded in paraffin wax. Sections of 3–4 µm were stained with haematoxylin and eosin (HE) and Van Gieson stain (for connective tissue fibres). General histopathological examination and photographic documentation was carried out at magnifications of 4×, 10×, 40×, and 100× with the objective lens and 10× greater with an eyepiece. The liver parenchyma, portal fields and cyst wall were examined. The morphometric analysis included individual layers of the cyst and bands of connective tissue in the parenchyma, which were measured at magnifications of 10× with the objective lens and 10× greater with an eyepiece. The microscopic evaluation was blind, using a standard BX41 light microscope and CellSens software (Olympus Corporation, Japan).

Results

Parasitological findings. Isolated trematodes and their eggs were identified as *P. fasciolaemorpha*. The infection was estimated to comprise 10,126 trematodes and the largest cavity to contain over 600. The parasites were located in the lumen of bile ducts which were dissected during necropsy. The isolated trematodes were small and leaf-shaped, and ranged from 2.9 to 7.5mm in length and 1.1 to 2.5mm in width. While the well-developed anterior end of the body had an oral sucker, the posterior end was sharply narrowed (Fig. 1).

Examination of faeces revealed the presence of 2,615 eggs in a 3 g sample. Isolated eggs ranged from 121.5 to 143 µm in length and 71.8 to 91.1 µm in width, and had light yellowish shells densely filled with yolk cells.

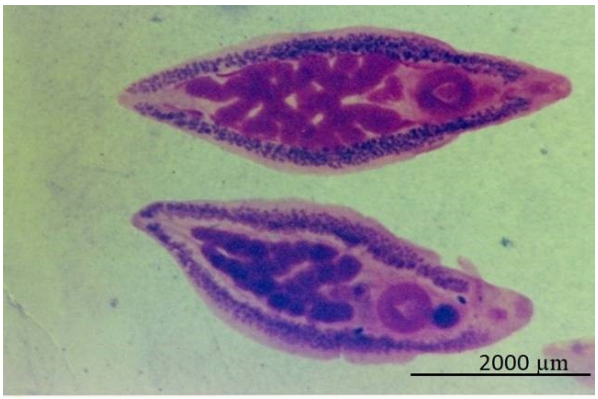

Fig. 1. *Parafasciolopsis fasciolaemorpha* flukes isolated from a moose liver

Pathomorphological changes. External examination revealed an enlarged, firm liver with rounded edges and a tender consistency. On the surface, yellowish, rounded spots with a diameter ranging from 1.0 to 3.0 cm were visible. In cross section, thickening of the bile ducts and multiple cavities connected to them were observed in the liver parenchyma. The cavities had a smooth, bright interior and were filled with dark brown liquid containing trematodes, their eggs, and cellular detritus. The cross-section of the liver also revealed a marked lobular pattern not visible in a healthy organ. The perimeter of the lobules was slightly lighter in colour with a greyish hue compared to the dark-brown central parts of the lobes.

Histopathological evaluation revealed numerous cavities in the liver parenchyma containing flukes and cellular detritus. The flukes were histologically identified on the basis of the structure of the cuticle, gastrointestinal tract, and ovaries (4, 18).

The cavities were separated from the liver parenchyma by a connective capsule with a layered structure (Fig. 2). Adherent cell detritus and exfoliated damaged and necrotic epithelial cells were found along

the lumen of cavities. A second layer 0.18 ± 0.014 mm wide was formed from dense connective tissue low in cell content with a few small blood vessels. A 0.19 ± 0.08 mm wide infiltration of inflammatory cells composed of histiocytes (containing giant cells), lymphocytes, and granulocytes with a large amount of eosinophils was also visible, as were hyperplastic bile ducts. The outermost layer was also the thickest (1.37 ± 0.03 mm width). It was formed by compact connective tissue fibres similar to dense regular connective tissue. The layer contained a small number of cells, with relatively large arteries and veins sequestered between connective tissue fibres that partially clamped the vessel lumens. The tunica intima of the arteries was also found to be thicker, probably as a result of smooth muscle hyperplasia.

Behind the cavities, strong hyperplasia of the connective tissue could be seen branching off the portal fields and forming bands 0.56 ± 0.06 mm wide around the liver lobules, extending between adjacent portal fields and occasionally present in intralobular areas (Fig. 3).

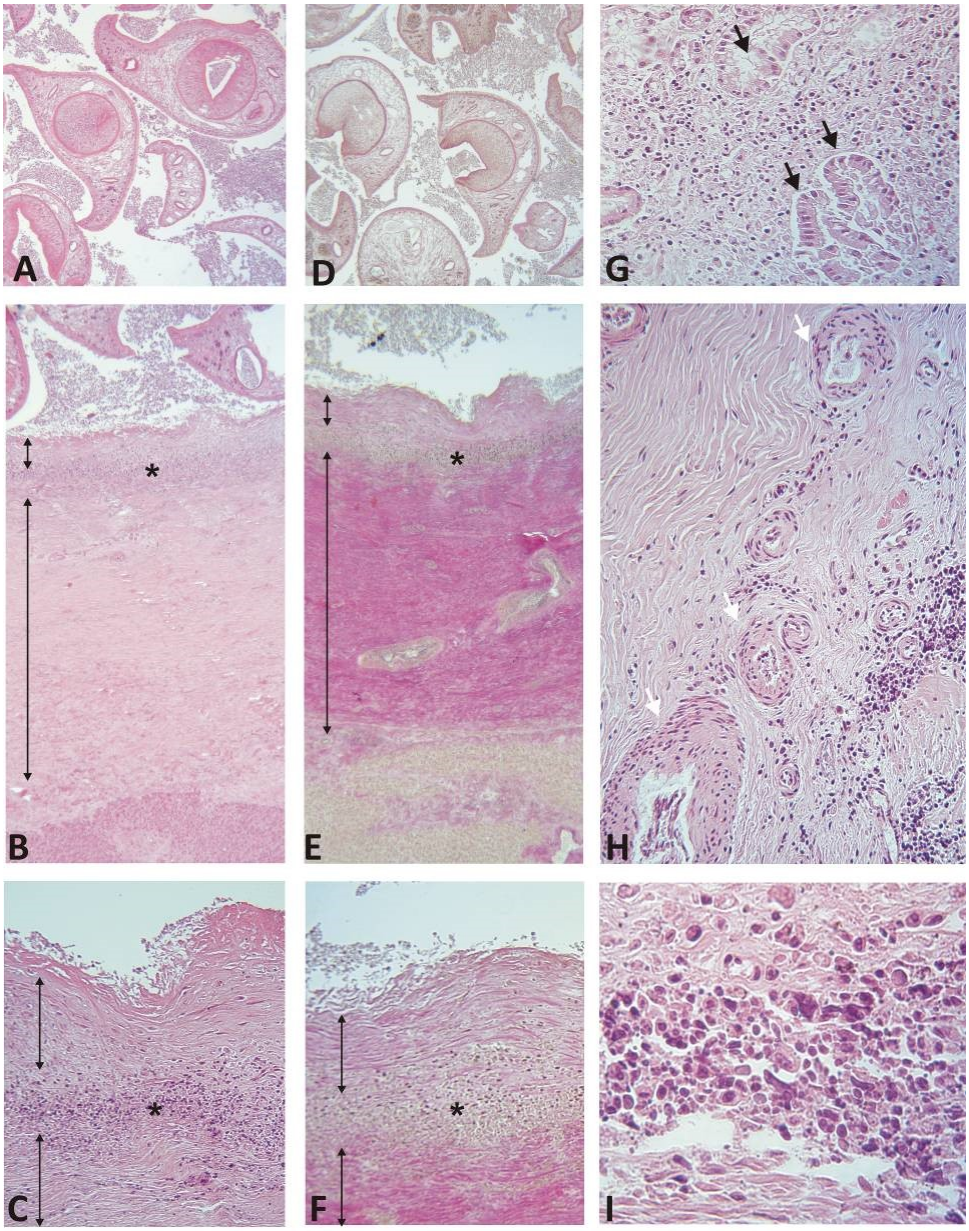

Fig. 2. Histopathological picture of cavities filled with flukes. A, D – liver flukes of *Parafasciolopsis fasciolaemorpha*, 10× (eyepiece) and 4× (objective); B, E – cyst capsule, 10× (eyepiece) and 4× (objective); C, F – the inner part of the cyst capsule, 10× (eyepiece) and 10× (objective); G – bile ductules in the layer of infiltration and secondary fibrosis, 10× (eyepiece) and 40× (objective); H – arteries in the layer of secondary fibrosis, 10× (eyepiece) and 40× (objective); I – cells of inflammatory infiltration, 10× (eyepiece) and 100× (objective). A, B, C, G, H, I – HE staining; D, E, F – Van Gieson staining. Black arrow – bile ductule, white arrow – artery, double arrow – connective tissue hyperplasia, * inflammatory infiltration

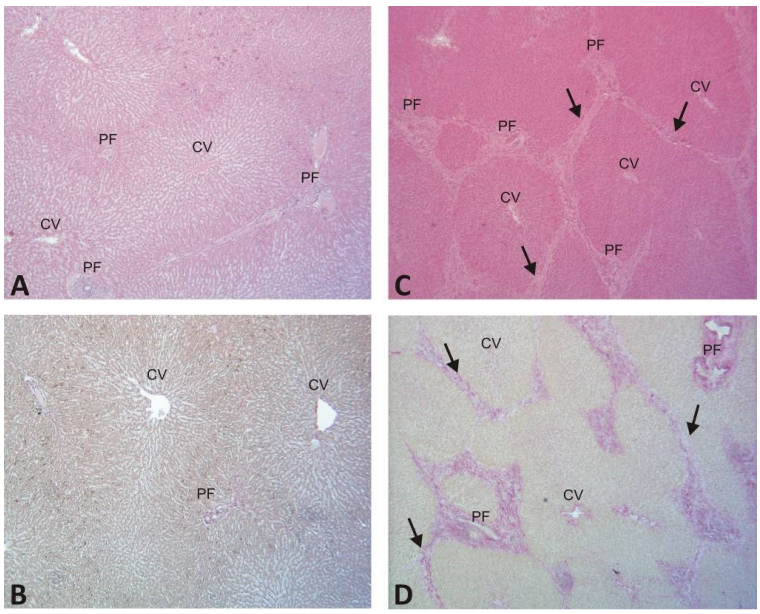

Fig. 3. Histopathological changes in the liver parenchyma in the infection with *Parafasciolopsis fasciolaemorpha* in comparison to the healthy moose liver. A, B – healthy moose liver; C, D – the parenchyma of a moose liver infected with *Parafasciolopsis fasciolaemorpha*; All images at 10× (eyepiece) and 10× (objective). A, B – HE staining; C, D – Van Gieson staining. Black arrow – connective tissue bands, CV – central veins, PF – portal fields

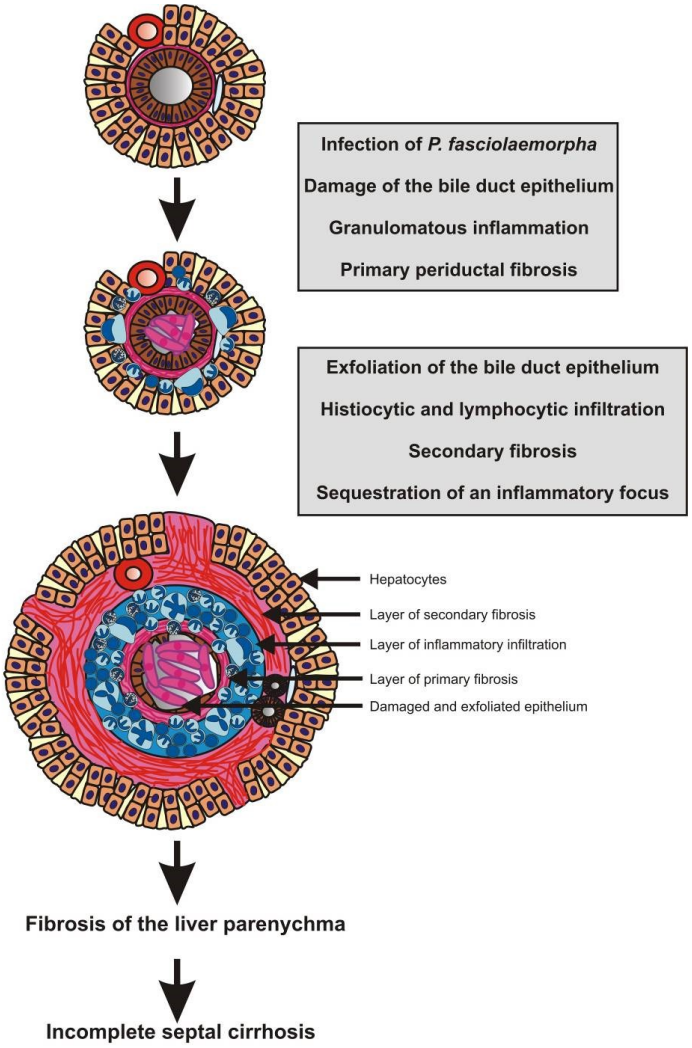

Fig. 4. Diagram of pathogenesis of *P. fasciolaemorpha* infection

In most lobules, the trabecular pattern of hepatocytes and the location of the central vein were preserved. No changes in the vessels or the bile ducts of the portal fields were visible. Hyperplasia around the central veins, single diffuse foci of intralobular fibrosis, and pseudolobuli without portal fields were sporadically observed, suggesting the presence of mild incomplete septal cirrhosis. However, no inflammatory infiltration was observed in the bands of connective tissue between the hepatic lobules. The changes observed in the liver suggested the pathogenesis of infection with *P. fasciolaemorpha* (Fig. 4).

Discussion

All pathological changes observed in the liver of the infected moose could be attributed to an extremely high intensity of infection and the presence of numerous flukes in biliary ducts and ductules. Our results confirm those of Manga-González and González-Lanza (12), who reported that the number of heavy pathological lesions increases with the intensity of infection. Previous studies have also found gross lesions to be heavily present in the livers of moose with parafasciolopsosis (1, 4, 18). However, no histological examination of moose infected with *P. fasciolaemorpha* has previously been performed and no information is available.

Histopathological examination of the liver revealed the presence of numerous cavities filled with flukes and cellular detritus, and covered with a layered capsule of connective tissue. The cavities were probably formed as a result of distension of the bile ducts obstructed by thousands of flukes. The subsequent cholangitis and cholangiectasis, which are also characteristic of infection with other trematodes (9, 11), resulted in the necrosis and exfoliation of epithelial cells of the bile ducts. The thick layer of connective tissue observed in the samples, which encysted damaged bile ducts and isolated flukes from the liver parenchyma, may have developed as a reaction to the obstruction of the bile ducts. This is because obstruction was found to provoke fibrosis and collagen formation (16). Similar fibrous cysts in the liver have previously only been observed following infection with the giant liver fluke *Fascioloides magna* (15). However, *F. magna* infection was a cause of serious damage to the liver parenchyma (6), which was not observed in parafasciolopsosis. Histopathological examination of the liver parenchyma revealed only hyperplasia of connective tissue, which also may have developed as a reaction to damage of the bile ducts. Similar portal fibrosis caused by activated hepatic stellate cells was observed in infection with *Dicrocoelium dendriticum* (9).

The low severity of the pathological lesions in the liver may be due to the fact that *P. fasciolaemorpha* migrates up to the biliary ducts and does not penetrate the liver parenchyma, unlike *F. magna* or *F. hepatica* (6, 13). However, the initial signs of incomplete septal cirrhosis following sporadic intralobular fibrosis were already visible, together with the absence of a central vein and the presence of an abnormal lobular structure. It is possible that moose with parafasciolopsosis are able to survive the infection by responding with extensive liver fibrosis, isolating thousands of flukes from the surrounding parenchyma and thus delaying the inevitable liver failure.

Changes observed during the present study in the liver of a moose did not indicate liver failure. Nevertheless, the very presence of flukes in the bile ducts and the following cholangiectasis and bile duct obstruction may have contributed to the diarrhoea and poor condition of the examined animal. Therefore, parafasciolopsosis with accompanying diarrhoea, resulting in emaciation and dehydration, was the most probable reason for the moose's death.

It is the second case of such intensive moose parafasciolopsosis in the Polesie region of eastern Poland identified during last two years (4). The rise in the local moose population (14) is linked to increases in the numbers of fatal parafasciolopsosis cases and the risk of infection to other ruminants. Although *P. fasciolaemorpha* infection does not appear to be associated with any serious reduction of the moose population in Poland, it is still a parasite of high veterinary importance because of its danger to wild cervids and domestic ruminants. Therefore, it is crucial to determine the effect on the definitive host of the extremely intensive infection which *P. fasciolaemorpha* gives rise to and which is unusual in other trematodes. This will allow a better understanding of the patterns of *P. fasciolaemorpha* transmission and possible scenarios of its spread in the environment. Further studies are needed to determine the prevalence of *P. fasciolaemorpha* in the moose population in Poland, the level of infestation of the environment with larval forms of the fluke, and the risk to other ruminants of parasitic infection.

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

Animal Rights Statement: None required.

Financial Disclosure Statement: This research did not receive any specific funding from agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments: The authors would like to thank the management and staff of Polesie National Park for assistance in animal necropsies and material collection. This study is a part of a PhD thesis concerning endoparasites of moose in Poland.

References

1. Dróżdż J.: Natural centre of parafasciolopsosis in the Białystok Province. Wiad Parazytol 1963, 9, 129–132.
2. Dróżdż J.: Studies on helminths and helminthiases in Cervidae II. The helminth fauna in Cervidae in Poland. Acta Parasitol Pol 1966, 14, 1–13.
3. Filip K.J., Demiaszkiewicz A.W.: Internal parasitic fauna of elk (*Alces alces*) in Poland. Acta Parasitol 2016, 61, 657–664.
4. Filip K.J., Pyziel A.M., Demiaszkiewicz A.W.: A massive invasion of *Parafasciolopsis fasciolaemorpha* in elk (*Alces alces*) in Lublin Province, Poland. Ann Parasitol 2016, 62, 107–110.
5. Islam K.M., Islam M.S., Rauf S.M.A., Khan A., Adhikary G.N., Rahman M.: Surveillance and pathology of fascioliosis (*Fasciola gigantica*) in black Bengal goats in Bangladesh. J Anim Sci Adv 2016, 6, 1837–1845.
6. Karamon J., Larska M., Jasik A., Sell B.: First report of the giant liver fluke (*Fascioloides magna*) infection in farmed fallow deer (*Dama dama*) in Poland – pathomorphological changes and molecular identification. Bull Vet Inst Pulawy 2015, 59, 339–344.
7. Kazlauskas J., Shlejjkus P.: *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932 u losejj Litovskojj SSR. Acta Parasitol Lithuanica 1962, 4, 193–194.
8. King J.M., Roth-Johnson L., Dodd D.C., Newsom M.E.: The Necropsy Book. A Guide for Veterinary Students, Residents, Clinicians, Pathologists, and Biological Researchers, Internet-First University Press, New York, 2013.
9. Kukolj V., Aleksić-Kovačević S., Katić-Radivojević S., Knežević D.J., Jovanovića M.: The role and immunophenotypic characteristics of myofibroblasts in liver of sheep naturally infected with the lancet liver fluke (*Dicrocoelium dendriticum*). Vet Parasitol 2015, 208, 181–189.
10. Lachowicz J.: Development of *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932 from eggs derived from sheep. Acta Parasitol Pol 1988, 33, 169–175.
11. Manga-González M.Y., Ferreras M.C., Campo R., González-Lanza C., Pérez V., García-Marín J.F.: Hepatic marker enzymes, biochemical parameters and pathological effects in lambs experimentally infected with *Dicrocoelium dendriticum* (Digenea). Parasitol Res 2004, 93, 344–355.
12. Manga-González M.Y., González-Lanza C.: Field and experimental studies on *Dicrocoelium dendriticum* and dicrocoeliasis in northern Spain. J Helminthol 2005, 79, 291–302.
13. Martinez-Moreno A., Jiménez-Luque V., Moreno T., Redondo E.S.H., Martin de las Mulas J., Pérez J.: Liver pathology and immune response in experimental *Fasciola hepatica* infections of goats. Vet Parasitol 1999, 82, 19–33.
14. Ratkiewicz M.: Strategia ochrony i gospodarowania populacją łosia w Polsce [Strategic plan for the protection and management of the Polish moose population (in Polish)]. NFOŚiGW, Białystok, 2011.
15. Swales W.E.: The life cycle of *Fascioloides magna* (Bassi, 1875), the large liver fluke of ruminants in Canada with observations on the bionomics of the larval stages and the intermediate hosts, pathology of fascioloidiasis magna, and control measures. Can J Res 1935, 12, 177–215.
16. Tahan G., Akin H., Aydogan F., Ramadan S.S., Yapicier O., Tarcin O., Uzun H., Tahan V., Zengin K.: Melatonin ameliorates liver fibrosis induced by bile-duct ligation in rats. Can J Surg 2010, 53, 313–318.
17. Taylor M.A., Coop R.L., Wall R.L.: Veterinary Parasitology. Blackwell Publishing, Oxford, 2007, pp. 799–800.
18. Wiśniewski L.W.: Experimental studies on the development of *Parafasciolopsis fasciolaemorpha* Ejsm. Reports of Warsaw Scientific Association 1936, 4–6, 119–149.