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HISTOPATHOLOGICAL CHANGES IN LIVER OF ELKS WITH *PARAFASCIOLOPSIS FASCIOLAEMORPHA* INVASION

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Abstract
Parafasciolopsosis is a parasitological disease which is caused by the liver fluke *Parafasciolopsis fasciolaemorpha.* This parasite which belongs to herbivores often causes invasion in elks (*Alces alces*). The aim of our study was to diagnose the parasite and investigate what kind of histopathological changes it caused in the liver tissue. The study took place in Latvia University of Life Sciences and Technologies, Faculty of Veterinary Medicine, Laboratory of Comparative Pathology and Laboratory of Parasitology. During the study from 2017 to 2018 we collected liver samples and faeces from 46 felled elks of different age and gender, from all over Latvia. Ten grams of faeces were examined for trematode eggs by sedimentation method and a section of liver was examined for the presence of flukes and the histological structure of the liver. In this study *Parafasciolopsis fasciolaemorpha* were found in 11 samples of liver from 46 elks, with invasion extensity of 24%. The histopathological examination of the liver samples revealed fibrosis, proliferation of bile ducts, pericholangitis, calcerous deposits in bile ducts and other notable histological changes in the liver tissue that can cause hepatic trematodes.
Key words: Parafasciolopsosis, *Alces alces*, hepatic trematode, liver tissue, faeces.

Introduction

Parafasciolopsosis is a parasitological disease caused by the liver fluke *Parafasciolopsis fasciolaemorpha* from the family *Fasciolidae*. It is a typical parasite of elks (*Alces alces*): however, the fluke has also been recorded in roe deer (*Capreolus capreolus*), bison (*Bison bonasus*) and other ruminants (Filip, Pyziel, & Demiaszkiewicz, 2016).

The agent was first diagnosed in 1932 in Eastern Europe (Filip & Demiaszkiewicz, 2016). Nowadays the disease is prevalent in central Europe as well as in the eastern and southern parts (Filip, Pyziel, & Demiaszkiewicz, 2016). Scientific publications show that the invasion is a topical issue in Poland, Hungary, Belarus, and Russia (Eckert *et al*., 2005; Filip, Pyziel, & Demiaszkiewicz, 2016). There are no international reports about the situation in the Baltic States.

This hepatic trematode has been diagnosed at the Faculty of Veterinary Medicine since 1970 by laboratory testing of liver samples from hunted elks. The epizootic data about parafasciolopsosis in Latvia present that elks are infected throughout the country (Bergmane *et al*., 2017). Therefore, research about parasite induced pathological changes in liver tissue and pathogenesis of the disease is topical. Due to the harmful effects of parasites, the infected elks have a growth disorder that is an important factor in the process of obtaining high-quality hunting trophies (Bergmane *et al*., 2017).

A mature fluke is lancet-like 3-7 mm in length and 1.0 – 2.5 mm in width (Eckert *et al*., 2005). The parasite locates in the bile ducts of the liver, and also in the duodenum and pancreas when the invasion is extremely intensive. An invasion can cause anemia, reduce body weight and productivity, growth inhibition in young animals and significant illnesses which may lead to falls (Filip, Pyziel, & Demiaszkiewicz, 2016).

The life cycle of the parasite is typical for the *Fasciolidae* family, which is mediated by an intermediate host – aquatic snail (Eckert *et al*., 2005; Filip *et al*., 2016). Animals infest after eating metacercaria, an invasive larvae usually found in water or on plants near water reservoirs (Eckert *et al*., 2005).

A juvenile fluke penetrates the wall of the duodenum and migrates to the liver where it matures. Liver damage and haemorrhage resulting in necrosis is associated with the migration of immature flukes through the liver. Adult flukes reside in the bile ducts where they produce eggs and cause cholangitis. As a result, the liver becomes enlarged, has multiple cavities filled with dark brown liquid, trematodes and eggs (Filip, Pyziel, & Demiaszkiewicz, 2016; Taylor, Coop, & Wall, 2016; Zachary, 2017).

Taking into account the actuality of parafasciolopsosis in Latvia, the aim of our study was to diagnose the parasite and investigate what kind of histopathological changes it caused in the liver tissue.

Materials and Methods

The research took place in Latvia University of Life Sciences and Technologies, Faculty of Veterinary Medicine, Laboratory of Comparative Pathology and Laboratory of Parasitology during the hunting seasons 2017 and 2018 (October – December). Liver samples from 46 felled elks and 34 faecal samples from the same elks of different age and gender, which were hunted in accordance with legislative acts, were included in this research. The samples, collected from all over Latvia, after examination results were divided into two groups: one was the negative group – 35 samples and the other was the positive group – 11 samples invaded with *Parafasciolopsis fasciolaemorpha.* The negative group was defined as a control group. Samples of liver

and faeces collected from the rectum were transported refrigerated to the laboratory for further examination.

Several methods have been used for parasitological examination of the faecal samples. Ten grams of faeces were examined for the presence of trematode eggs by sedimentation method (Zajac & Conboy, 2006). McMasters method was applied for qualitative testing of the presence of other helminthes eggs base of gastrointestinal nematode (Bowman, 2013). The Baermann test was used to isolate larvae from faecal samples, mostly to diagnose lungworm infections (Zajac & Conboy, 2006). The rate of infestation of animals was estimated by calculating the invasion extension. Considering that faecal samples were not collected from all 46 elks, the samples from the liver also were examined for the presence of flukes by the clinical examination where flukes were found in parenchyma filled in multiple cavities and ducts and visually as lancet like organisms (Filip, Pyziel, & Demiaszkiewicz, 2016).

The samples of liver for histological examination were fixed in 10% formalin solution until processing into paraffin blocks. For the histological tissue visualization samples were prepared by standard Hematoxylin and Eosin (H&E) staining method and later examined under a light microscope in 100, 200 and 400 time magnification to evaluate microscopic tissue changes such as fibrous tissue formation, bile duct changes, etc. (Zachary, 2017).

Results and Discussion

The first task of this research was to find appearance of parasites during parasitological examination. Results of the coprological studies show that from 34 investigated faecal samples *Parafasciolopsis fasciolaemorpha* was detected in eight samples with invasion extension of 24%.

The results of the 46 liver clinical examinations show 11 positive cases – eight from the same elks, which have positive faecal samples, two samples with the negative faecal examination and one without the faecal examination. In general, our study shows that *Parafasciolopsis fasciolaemorpha* were found in 11 samples from 46 elks, with invasion extensity 24%.

Higher prevalence of parafasciolopsosis is reported by researchers from Poland, where it is widespread. Several studies have found that the extent of the infestation of parafasciolopsosis reaches 69% – 100% (Filip, Demiaszkiewicz, 2016; Filip *et al*., 2016). In contrast, in other studies carried out in North America (Bildfell *et al*., 2007; James & Maskey, 2011) and Norway (Davidson *et al*., 2015), where the elk population is dense, trematode *Parafasciolopsis fasciolaemorpha* was not detected. In a study conducted in Lithuania in 2007 to detect internal parasitic fauna of elks, trematode *Parafasciolopsis fasciolaemorpha* was not detected, though *Fasciola hepatica* was detected (Davidson *et al*., 2014; Aukštikalniene *et al*., 2007; Bildfell *et al*., 2007; James & Maskey, 2011).

For more appropriate evaluation of invasion extension of elk parasitosis in Latvia future investigation will be performed.

According to coprology study results, other parasites were found such as *Trichostrongylus* spp. with invasion extensity 88%, *Trichuris* spp. – 73%, *Strongyloides* spp. – 35%, *Protostrongylus* spp. – 27%, *Moniezia* spp. – 12% and *Paramphistomum* spp. – 6%. The fact that nematodes are more frequent than the presence of trematodes of *Cervidae* also is confirmed by other studies in Lithuania (Aukštikalniene, Bukelskis, & Kašetaite, 2007), in Poland (Filip & Demiaszkiewicz, 2016) and in Norway (Davidson *et al*., 2015), where scientists found that the most common nematode of elks was *Trichuris* spp. and of deers – *Strongyloides* spp.

The histopathological examination of the positive samples from the liver with *Parafasciolopsis fasciolaemorpha* invasion revealed proliferation of interlobular connective tissue detected as interlobular fibrosis, which surrounds liver lobules, vessels and bile ducts – was found in all positive cases, as well as biliary hyperplasia. For the control group the quantity of interlobular bile ducts located in the portal area average was three to five, while for the positive samples the number was greater by ten. Inflammatory cell infiltration of macrophages and neutrophils were seen in portal areas and around bile ducts, indicating pericholangitis. Some of the bile ducts were complete with calcerous deposits that can cause biliary obstruction. Previously named pathological changes in liver more improved dilatation of bile ducts (Filip, Pyziel, & Demiaszkiewicz, 2016).

Such pathological changes in the liver are also described by other hepatic trematodes: *Fasciola hepatica, Fasciola gigantica* and *Fascioloides magna* (McGavin, Carlton, & Zachary, 2001; Smith, 2015; Taylor, Coop, & Wall, 2016; Zachary, 2017). Mature flukes remain in the larger bile ducts and are the reason for cholangitis. Inflamatory infiltrate of neutrophils and macrophages is often found (Zachary, 2017). Normally interlobular connective tissue is poor and difficult to see (Eurell & Froppier, 2006), as it was for the control group. Interestingly, that with *Fasciola hepatica* infection duct calcify is characteristic to cattle but not sheep (Smith, 2015). The fact that calcium sediment is typical of parafasciolopsosis invasion in the bile ducts is confirmed by case report from Poland where it is mentioned that such deposits may completely block the bile duct (Filip, Pyziel, & Demiaszkiewicz, 2016). The researches show that bile duct obstruction can lead to extrahepatic cholestasis (McGavin, Carlton, & Zachary, 2001). Such changes

were not detected in this research. *Fasciolides magna* is characterized by the formation of a black pigment, which is not detected in other flukes (Zachary, 2017).

Further studies are necessary to better understand the influence of the parasite not only on the liver but also on other tissues in the body.

Conclusions

The results of the research show that *Parafasciolopsis fasciolaemorpha* was found with invasion extensity 24%. Histological examination of the liver samples invaded with Parafasciolopsis fasciolaemorpha showed typical chronic cholangial changes similar to cases of other hepatic trematodes invasion.

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