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Original papers

A massive invasion of *Parafasciolopsis fasciolaemorpha* in elk (*Alces alces*) in Lublin Province, Poland

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ABSTRACT. Liver fluke *Parafasciolopsis fasciolaemorpha* is a parasite typical of elk (*Alces alces*). The present study describes the identification of a massive invasion of the liver fluke *P. fasciolaemorpha* in an elk in the Sobibór Landscape Park, Eastern Poland. In February 2016, samples of liver and faeces were collected from the elk during post-mortem examination. A section of liver tissue and three grams of faeces were examined for the presence of flukes or fluke eggs by decantation. In total, 11,150 juvenile and mature flukes of *P. fasciolaemorpha* were found in the examined liver. Multiple cavities with distinct walls, filled with dark liquid, trematodes, fluke eggs and cellular detritus were seen in the cross section of the parenchyma. Additionally, some of the bile ducts were plugged with calcerous deposits. The three grams of examined faeces were found to contain 322 grey and golden-coloured eggs of *P. fasciolaemorpha*. The presence of such a massive infection of *P. fasciolaemorpha* in the examined elk has a significant impact on the health of the individual. We can assume *P. fasciolaemorpha* has a significant influence on the health and abundance of the entire elk population in Poland.

Key words: *Parafasciolopsis fasciolaemorpha*, elk, Sobibór Landscape Park, Poland

Introduction

Liver fluke *Parafasciolopsis fasciolaemorpha* is a parasite typical of elks (*Alces alces*). The first natural focus of elk parafasciolopsosis in Poland was identified in Polesie [1], and this was followed by another in the Rajgród Forest district [2]. However, the fluke has also been recorded in roe deer (*Capreolus capreolus*) in the forest complex of Kielce [3] and in bison (*Bison bonasus*) from the Białowieża Forest [4]. The geographical range of *P. fasciolaemorpha* encompasses a large part of central and eastern, as well as southern, Europe.

Parafasciolopsis fasciolaemorpha occurs in extremely massive invasions in a natural definitive host, the elk, with a prevalence reaching 100% [2]. The intensity is also significant and can range from several thousand to several hundred thousand parasites per infected animal [2,5]. An invasion can result in reduced body weight, growth inhibition in young animals and serious illnesses which may lead to falls [6].

The life cycle of the parasite is typical of the Fasciolidae. Within four weeks, miracidia hatch from eggs excreted in the faeces. Miracidia actively penetrate the tissues of the aquatic snail (*Planorbarius corneus*) within the Planorbidae family. This species is considered to be the only intermediate host of *P. fasciolaemorpha* [5]. Miracidia in the snail tissue develop into another larval form, the sporocyst. Inside the sporocyst, one parent redia develops into embryonic balls, giving rise to a single daughter redia [7]. Each daughter redia gives rise to a cercaria, which actively emerges into the water and transforms into a metacercaria, the invasive form of the parasite, which can be accidentally swallowed with water or food by an animal. In the digestive tract of an elk, a juvenile fluke emerges from the metacercaria, penetrates the intestinal wall and migrates to the liver, where it develops to maturity. Mature flukes occur in the bile ducts of the liver, as well as in the duodenum and pancreas during an extremely intensive invasion.

The aim of this study was to determine the numbers of *P. fasciolaemorpha* present within an infected elk in the Sobibór Landspace Park, Eastern Poland. This animal is the first elk inspected for the presence of parafasciolopsosis in Lublin Province. Our findings will not only broaden knowledge of the biology of *P. fasciolaemorpha* but also of the health status of elk in Lublin Province. Further studies of parafasciolopsosis in wild ruminants in this region of Poland are being planned.

Materials and Methods

A wounded elk, identified as a two-year-old female, was found in February 2016, in Sobibór Landscape Park (Wołczyny Forest district). The animal was euthanized because of serious injuries. A field necropsy was performed two days later. Samples of liver tissue and fecal samples from the rectum were collected and transported to the laboratory for further investigation.

The liver was subjected to external examination and then was dissected by repeated sections, giving small pieces of liver, which were examined for flukes by decantation. The flukes were isolated from the sediment and counted under the light microscope (16×magnification) in Petri dishes. Three grams of faeces samples were also examined for trematode eggs by decantation, with the eggs being counted in small Petri dishes under the light microscope (25× magnification).

Results and Discussion

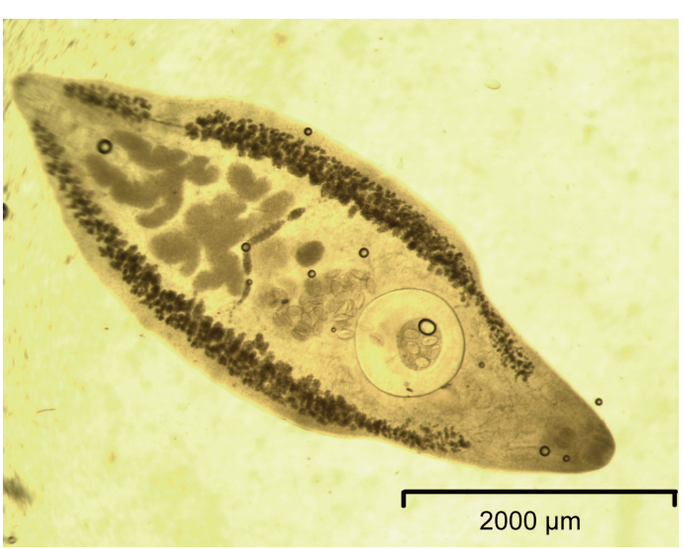
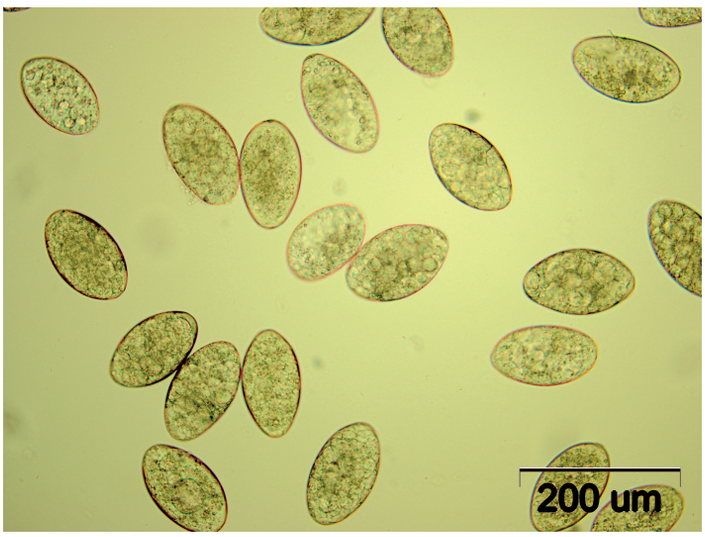


Fig. 1. Fluke *Parafasciolopsis fasciolaemorpha* found in the liver

A total number of 11,150 juvenile and mature flukes of *P. fasciolaemorpha* were found in the examined liver (Fig. 1). Many flukes were juvenile, not fully mature. *P. fasciolaemorpha* is highly pathogenic for its natural host and causes significant pathological lesions in the liver [8], which were also observed in this case. The surface of the organ was tensed and grey in colour, while the organ itself was enlarged with rounded edges and a tender consistency. In cross section, the parenchyma was found to have multiple cavities with distinct walls, filled with dark brown liquid, trematodes, fluke eggs and cellular detritus (Fig. 2). These cavities (pseudocysts) were connected with bile ducts. Some of the bile ducts were plugged with calcerous deposits. Such deposits may sometimes completely block the bile duct [5].

Parafasciolopsis fasciolaemorpha flukes occur in the bile ducts of the liver, and during intensive invasions, may cause clogging of the bile ducts, as

Fig. 2. Pseudocyst in the liver parenchyma



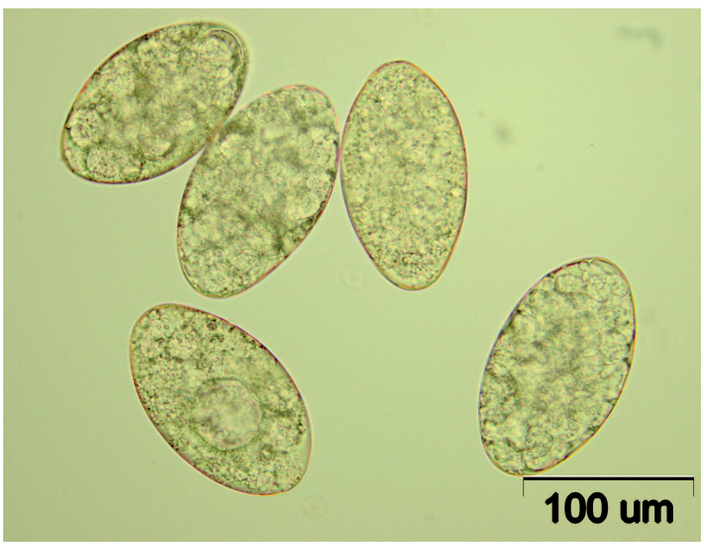


Fig. 3-4. Eggs of *Parafasciolopsis fasciolaemorpha*

well as the extension and consequent formation of cavities filled with parasites and liver tissue breakdown products [9], as observed in the case described herein. In total, 322 grey and golden-coloured eggs were found in three grams of faeces (Fig. 3,4). It is possible to differentiate the eggs of *P. fasciolaemorpha* from those of other elk flukes (*Fasciola hepatica* and *Paramphistomum cervi*) [7] based on their shape: The eggs of *P. fasciolaemorpha* have a different shape to those of *F. hepatica* and *P. cervi* and are more squat. Their structure is less granular and are coloured grey and golden, in contrast to the golden eggs of *F. hepatica* and the grey eggs of *P. cervi* [7].

Despite the fact that many of the flukes found in the liver were juvenile, many others were fully mature and producing eggs. Their numbers found in the faeces confirm the very high level of invasion. Eggs and mature flukes were also observed in the liquid filling the cavities (pseudocysts) found in the liver. Some of them started to develop after few days in the conditions of the fridge but no miracidia were observed.

Such an intensive invasion of liver flukes caused significant gross lesions in the liver, which probably affected the health of the infected animal. It is known that *P. fasciolaemorpha* causes lack of appetite, malnutrition and growth inhibition in the definitive host, which is very dangerous, especially for young animals, and may lead to falls [6].

This fluke always occurs in natural hosts in massive invasions. The highest number of flukes found in one examined animal was 117,800 parasites [10]. A significant invasion of *P. fasciolaemorpha* was also reported in elks in Belarus, with 27,500 flukes being identified in one animal [11]. However, in another study, only two of a group of ten elks investigated in Russia near Moscow were found to be infected with *P. fasciolaemorpha* (intensity three to seven flukes), which gives very low intensity and prevalence [12].

There is close relationship between the ecology of the elk and aquatic snail (*Planorbarius corneus*), which is the only one known intermediate host of *P. fasciolaemorpha*. Both elk and snail live in a marshy environment in great densities. Cercariae emerge from snails for most of the year with a peak in June. The large number of larvae present in snails in this period occurs as a result of a fluke invasion from the previous season. Under laboratory conditions, it was found that the period of larval development in the intermediate host is 197 days in autumn and winter, and only 96 in the summer [13]. Lachowicz [7] notes that there could be as many as 252 developing larvae of *P. fasciolaemorpha* in a snail at the same time. This relationship between the elk, as a definitive host, and the snail, as an intermediate host, can account for the enormous intensity of infection observed in the natural definitive host and prevalence reaching 100%.

The presence of *P. fasciolaemorpha* in elks is a potential threat to domestic animals. Under laboratory conditions, sheep are very susceptible to infection by this fluke. In cattle, experimental infection is also possible [1]. However, a study of 150 cows from a slaughter house in Ełk and three from another slaughter house in Rajgród, the region where 100% of elks were infected with *P. fasciolaemorpha*, found none of the domestic animals to be infected, despite the fact that cows

were grazing on marshy areas which were often shared with elks [7].

Although parafasciolopsosis seems not to be a threat for domestic animals under standard conditions, the infection should be monitored in wild ruminants. The high level of *P. fasciolaemorpha* infection observed in a single elk in Lublin province suggests the presence of these flukes in this region in great numbers. More animals from this region should be examined to determine the prevalence of parafasciolopsosis, and to compare it with results obtained from other areas tested. The numerous wetlands present in the Lublin province appear to be the ideal environment for *Planorbarius corneus*: the intermediate host of *P. fasciolaemorpha*. However, the occurrence of larval forms of *P. fasciolaemorpha* in *Planorbarius corneus* in Lublin province still remains unknown and further studies are required in this area.

Due to the number and variety of gross lesions associated with *P. fasciolaemorpha* infection in the dissected liver of the elk, we can assume that that parasite had a great influence on the health of the host. Bearing in mind the significant impact of parafasciolopsosis on the health and abundance of the elk population in Poland, further studies are needed to better understand its extent.

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