Case Report

Severe pulmonary fascioloidosis in a wild Mouflon (*Ovis musimon*) - a case report

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Summary

A wild male mouflon (*Ovis musimon*) was shot due to the observed weakness. Necropsy revealed consolidated lungs and traces of black pigment and fibrin on the liver. On the cut surface, a juvenile fluke was found in the lungs, while traces of destroyed flukes' migratory channels were found in the liver. *F. magna* infection in both, wild and domestic ruminants, causes three types of species-specific host-parasite interactions; definitive, dead-end and aberrant. Mouflon are classified as aberrant hosts and here we report unsuccessful migration of a juvenile fluke that led to a severe pneumonia.

Keywords: Mouflon; fascioloidosis; unsuccessful migration; pathology; aberrant host

Introduction

In Europe, the detrimental effects of fascioloidosis are considered prominent with regard to roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) populations. Interestingly, the responsibility for the introduction of a disease primarily associated with North American deer species, lies with the deer farmers, wildlife managers and hunters (Rehbein *et al.*, 2021) for whom it now causes concern. With the aims of improving the herds of native red deer, aesthetic reasons (for parks) and for hunting purposes (Rehbein *et al.*, 2021) mainly white-tailed deer (*Odocoileus virginianus*) and wapiti (*Cervus elaphus canadensis*) were imported into nineteenth century Europe. Along with them, a non-native digenean trematode *Fascioloides magna* was introduced. Its detrimental effect was first described in 'La Mandria Park' in Italy, but did not spread further due to the existing fence (Krállová-Hromadová *et al.*, 2016). Recent genetic studies confirmed that it was introduced to Europe at least twice, forming additional permanent foci in the Czech Republic and Danubian

floodplains. After the reports of fascioloidosis in Germany, Austria, Hungary, the Slovak Republic, Croatia and Serbia (for a review see Krállová-Hromadová *et al.*, 2016), the connection between its spread and the path of the River Danube was firmly established by genetic confirmation (Bazsalovicsová *et al.*, 2013).

In Europe, *F. magna* infects three types of final wild hosts, definitive (red and fallow deer) aberrant (roe deer and mouflon and experimentally chamois) and dead-end (wild boar). Cases of fascioloidosis in mouflon are known from previous field observations, but are rarely reported in the scientific literature (Janicki *et al.*, 2012; Konjević *et al.*, 2018). The line of events and outcomes of invasion are disparate in different types of hosts. In aberrant hosts juvenile flukes penetrate the intestinal wall and migrate along the peritoneal cavity in search of the liver. In cases when parasites reach the liver, pseudocysts are rarely formed and the continuous migration of flukes incite considerable liver damage leading to high mortality (Kralová-Hromadová *et al.*, 2016).

Here we present the lesions observed in a five-year-old wild mouflon with severe pneumonia caused by the migration of

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Fig. 1. Part of the mouflon liver. Note fibrin deposits and black stained traces (iron-porphyrin).

disorientated juvenile *F. magna* to highlight the difference in the severity of lesions found in the final hosts compared to those in aberrant one.

Case presentation

A five year old wild male mouflon was shot in December 2020, in the open state hunting ground "Zapadna Garjevica" (continental Croatia) due to its obvious weakness and inability to climb uphill. Hunting ground Zapadna Garjevica is situated partly in Bjelovarsko-bilogorska County and partly in Sisačko-moslavačka County (continental part of Croatia). It covers a total area of 25529 ha. Main game species include red deer, fallow deer, roe deer, wild boar and mouflon. Combined prevalence on fascioloidosis during 2019 and 2020 in red deer, based on examination of livers collected from all shot animals was 62.5 % (n = 75/120). Disease control program with application of Tribix® (triclabendazole as active compound) on corn as a drug carrier was launched in winter of 2019 for red deer as main risk group for maintenance and spread of *F. magna*. Depending on the type of feeding places, other species occasionally could also have an access to the medicated feed. The age of the animal was estimated based on the count of horn annuli (Wagenknecht, 1984). A rapid onset of disease was suggested due to the animal's good body condition. The animal was eviscerated by game owners and complete viscera

was stored in a plastic box at +4 °C. Following the evisceration, a small black-stained area on the ribs was observed, indicative of iron porphyrin which is a by-product of *F. magna* metabolism. The internal organs were examined macroscopically and hence the lungs and liver were separated for further analysis due to the visible lesions. Other organs appeared to be normal. Samples of the lungs and livers were taken into 10 % buffered formalin and analyzed histologically using routine hematoxylin and eosin staining.

Liver

Macroscopically outer surface of the liver was covered with fibrinous deposits and traces of black pigment were visible (Fig. 1). Following external observations, the liver was cut on approximate 2 cm thick slices and thoroughly examined from both sides. Present within the liver parenchyma were pigment traces and focally small agglomerations of yellow stained debris (up to 0.5 cm in size) indicative of decaying fluke following ingestion of the trematode (consummation of medicated feed exposed for red deer). Histologically, the channels from the migrating flukes were visible on sections, varying in size from a few millimetres to 1.5 cm in diameter and were filled with necrotic debris composed of cellular karyorrhectic debris, degenerate neutrophils and eosinophils, fibrin and erythrocytes. Along the channels and multifocally (mainly in the periportal area), there were numerous

Fig. 2. Lungs, dorsal surface. Caudal lung lobes are longitudinally cut to reveal migratory channels of the *F. magna* fluke. The migratory channels are characterized by elongated haemorrhagic and necrotic areas (evident on both sides) and with occasional cavitations (right lobe, left side of the figure). The left cranial lung lobe has one round haemorrhagic focus with central rupture of pleura and likely presents an exit point of fluke's lung migratory pathway.

separate accumulations of iron porphyrin. In the periportal area, there was minor infiltration of lymphocytes and plasma cells in addition to bile duct hyperplasia.

Lungs

The lungs (which were the organ with the dominant findings in the thoracic cavity), were examined macroscopically, by palpation and on the cross section. The colour of the lungs varied from reddish brown to yellow. In the yellow part of the lungs, a circular opening was observed, potentially indicating a point of entry of a fluke. On the cut surface, consolidation of the parenchyma was visible with numerous haemorrhages and openings corresponding to *F. magna* migratory channels (Fig. 2). Under compression, one juvenile fluke (measuring approximately 2 cm in length) was removed from the lungs (Fig. 3). Histologically, the majority of the lung parenchyma was replaced with necrotic areas which were surrounded by macrophages and fibrous tissue (capsule) containing activated fibroblasts. Within the necrotic centre, there was a large amount of fibrin, haemorrhages and remnants of dead flukes. Within the fibrous capsule and in the surrounding lung parenchyma, extensive hematin pigment was observed. In the areas without migratory channels, the alveolar septa were widely separated by inflammatory infiltrate containing; neutrophils, eosinophils, lymphocytes and macrophages (Fig. 4 – 5.). Incidentally, some of

Fig. 3. Juvenile fluke removed from the mouflon lungs.

Fig. 4. Lung, a segment of the migratory tract (channel) of *F. magna*. There is a mixture of fibrin, extravasated erythrocytes, and necrotic debris within the centre of the migratory tract (most of the lower half of the figure). Surrounding the centre (upper half of the figure) there is an elaborate immature fibrous tissue entrapping a few smaller and collapsed airways, and infiltrated by moderate numbers of lymphocytes, plasma cells, macrophages, and fewer neutrophils and eosinophils. Note numerous dark brown iron porphyrin pigment granules within the cytoplasm of the macrophages. Hematoxylin and eosin stain, 40X total magnification.

the bronchioles also contained adult nematodes and embryonated oval nematode eggs within the alveoli.

Discussion

A sanitary hunt of poorly conditioned red deer in the Šeprašhat region in Eastern Baranja led to the first report of fascioloidosis in Croatia (Marinculić *et al.*, 2002). The spread of *F. magna* to eastern Croatia has been attributed to both migration of affected red deer from Hungary and to favourable conditions of Danubian river plains (Marinculić *et al.*, 2002). In the subsequent years' disease have spread over the major part of the middle and eastern Croatia. The life cycle of *F. magna* resembles the one of *F. hepatica*, involving intermediate hosts like *Galba truncatula* or *Radix peregra* snails and stages such as miracidium, redia, cercaria and metacercaria. Metacercaria can be found on vegetation waiting to be consumed by the final host (Králová-Hromadová *et al.*, 2016). Following the ingestion of the metacercariae, juvenile flukes penetrate the

intestinal wall and enter the peritoneal cavity (Králová-Hromadová *et al.*, 2016). Under the normal circumstances juvenile flukes then travel through the peritoneal cavity in search of the liver, penetrate the Glisson's capsule and migrate through the liver parenchyma, avoiding the immune responses of the host. Upon completion of migration a pseudocyst can be formed. It is generally believed that migratory flukes are searching for the liver based on the chemical cues, despite the previous beliefs that parasites rely only on random wanderings (Dawes & Hughes, 1964; Sukhdeo *et al.*, 1987). This is partially understandable if known that chemical-based orientation is seen as chemotaxis and chemokinesis. Chemokinesis, being a multidirectional random movement, is a less optimal way of orientation and bears a potentially lower success rate of the migration process. Such characteristics of chemokinesis have potentially led to previous conclusions that fluke migration was based on a random wandering. Regardless of whether they use chemotaxis or chemokinesis the success rate of migration, based on *Fasciola hepatica* research, is usually

Fig. 5. Lung, a segment of one smaller migratory tract (channel) of *F. magna*. Adjacent to lumen filled with erythrocytes there is a narrow layer of degenerated and necrotic inflammatory cells which is further bounded with an immature fibrous tissue of similar composition as described in figure 2. Note one medium-sized bronchiole and several collapsed and distorted airways which are filled with cellular and karyorrhectic debris and extravasated erythrocytes (left half of the figure). Hematoxylin and eosin stain, 100X total magnification.

less than 50 % (Montgomerie, 1928). Following such accidental migration flukes would usually reverse their direction in search for the target organ. According to Sukhdeo *et al.* (1987) such line of events indicate that both of these species can recognize target tissue only upon entering it. This is especially emphasized in cases of aberrant hosts where a disorientated fluke has been seen more frequently to migrate to other organs (such as the spleen or lungs) and cause considerable damage. In such organs mechanical damage is intensified by inflammation due to intestinal bacteria carried by flukes. In a domestic sheep, a close relative of mouflon, development of *F. magna* infection was mainly observed under the experimental (Foreyt & Todd, 1976; Stromberg *et al.*, 1985; Foreyt, 1990) and less frequently under the natural conditions (Campbell & Todd, 1954; Foreyt & Hunter, 1980). In all, except one case, *F. magna* infection proved to be lethal for sheep. Gross lesions were mainly observed in the livers, and less frequently in kidneys, spleen and lungs. Only in one case, the infected sheep survived for a prolonged time and appeared to be healthy (Foreyt, 1990).

The animal was euthanised 8 months after the experimental infection and ten sexually mature flukes were recovered from livers, while eggs were found in liver parenchyma and unusually, also in the faeces. In experimentally infected bighorn sheep (*Ovis canadensis*), 42 flukes were recovered post mortally from three animals, of which only 2 were found in the lungs (Foreyt, 1996). All animals died within 197 days following the inoculation of metacercaria. Similar to our findings and findings in sheep, lung lesions were observed following experimental infection of Guinea pigs with *F. magna* (Conboy *et al.*, 1991). They described that although the liver was the most commonly affected organ, lung involvement was present in 72 % of infected guinea pigs 8 to 9 weeks post infection (Conboy *et al.*, 1991). Occasionally, disorientation can occur in other types of hosts, for example lung involvement has been described in definitive hosts of *F. magna*. Karamon *et al.* (2015) described pathological changes in a case of infected three-year-old fallow deer. Observed gross lesions included black spot pigmentation and inflammatory infiltrates in

the lungs (Karamon *et al.*, 2015). However, in this type of host flukes have left the lungs before inducing severe damage. Lee *et al.* (2016) performed a necropsy of a previously healthy 18 month old Brangus heifer (cattle represent a dead-end host). The main findings included exsanguination of the thoracic cavity, two large foci of pulmonary necrosis in addition to arteriolar and lung rupture due to *F. magna* infection. These findings prove that even though disorientation of flukes are possible following natural infection in all three types of hosts, it varies in extent and severity of lesions.

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Conflict of interest

Authors declare that they have no conflict of interest.

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