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Research Note

New data on wild boar (*Sus scrofa* L.) a dead-end host for large American liver fluke (*Fascioloides magna*)

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Summary

Fascioloidosis is a parasitic disease of primary wild and domestic ruminants, caused by a digenean trematode, *Fascioloides magna*. The final hosts of *F. magna* are divided according to the host-parasite interactions into definitive, dead end and aberrant. The clinical appearance, pathology, outcome of disease, and its importance in disease epidemiology vary with different host types. According to this division, wild boar (*Sus scrofa*) are characterized as a dead end host. In this paper we analysed 12 wild boar livers from Croatia. Eleven of them contained pigment traces, pseudocysts, degrading pseudocysts, fluke migratory channels, live and degrading flukes. *F. magna* eggs were found in pseudocysts, but no eggs were recovered from faeces. Concurrent infection with *F. magna* and *Fasciola hepatica* was detected in one liver. According to everything we observed, wild boar currently has no direct role in maintaining and spreading the disease.
Keywords: wild boar; fascioloidosis; pathology; dead end host

Introduction

In wildlife medicine fascioloidosis is a serious or even fatal disease of primarily wild and domestic ruminants. Originally a disease of North American deer species, caused by a digenean trematode, *Fascioloides magna* (Bassi, 1875), fascioloidosis was described for the first time in Europe in the former Royal Park La Mandria near Turin in Italy (for a review see Králová-Hromadová *et al*., 2016). Subsequently, the disease was detected in the Czech Republic (Ullrich, 1930; Erhardová-Kotrlá, 1971), Germany (Salomon, 1932), Austria (Pfeiffer, 1983), Hungary (Majoros & Sztojkov, 1994), the Slovak Republic (Rajský *et al*., 1994, 2002), Croatia (Marinculić *et al*., 2002), and Serbia (Trailović *et al*., 2008). Apart from Italy and the Czech Republic, the observed spread of fascioloidosis is related to the path of the River Danube, as was recently confirmed genetically (Bazsalovicsová *et al*., 2013). Molecular analysis also showed that *F. magna* has not spread from Italy to the rest of the Europe, but that this parasite was introduced into Europe at least twice (Králová-Hromadová *et al*., 2011). In each of these countries, fascioloidosis has been recognized as a significant health problem of red deer (*Cervus elaphus* L.), but even more of roe deer (*Capreolus capreolus* L.), fallow deer (*Dama dama* L.) and mouflon (*Ovis musimon*), and has had a devastating effect on these populations. The classification of hosts regarding their relation to *F. magna* includes: definitive hosts, in which the parasite matures within the pseudocyst in the liver parenchyma (e.g. red deer, fallow deer); dead end hosts, in which the parasite reaches the liver but rarely matures, and eggs, if produced, are not shed to the environment (e.g. moose, sika deer, bison, cattle, horse, wild boar), and, finally, aberrant hosts, in which the formation of pseudocysts does not occur, leading to excessive tissue damage by constantly migrating flukes, and consequent high mortality (e.g. sheep, chamois, mouflon, roe deer) (Králová-Hromadová *et al*., 2016).

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Descriptions of fascioloidosis in wild boar (*Sus scrofa* L.) are rare in scientific literature, and findings differ between the available reports (Foreyt & Todd, 1972; Foreyt *et al*., 1975; Balbo *et al*., 1989; Schwartz *et al*., 1993; Giczi, 2008). For instance, Králová-Hromadová *et al*. (2016) mention wild boar as a rare dead end host. On the other hand, a 69 % prevalence of *F. magna* infection in feral pigs from Southern Texas was documented by Foreyt *et al.* (1975). In this paper we present gross and microscopic findings of wild boar fascioloidosis, and discuss its potential significance for maintaining and spreading the disease.

Material and Methods

Study area and animals

A total of 12 liver samples, accompanied by faecal samples, were collected from wild boar, shot during regular hunting operations on the open state hunting ground No. III/28 ''POSAVSKE ŠUME''. It is a typical lowland habitat, with altitudes varying between 92 and 105 m.a.s.l., covering a total surface of 12,236 ha. Hydrological conditions are influenced by the Sava, Sunja and Una rivers. There are several streams and marshlands. Most of the forests are floodplain forests of pedunculate oak (*Quercus robur*) and great green weed (*Genisto elatae*), or specific forests of pedunculate oak and common hornbeam (*Carpinus betulus*). These forests grow in lowland areas with relatively high groundwater (Vukelić, 2012). Such conditions have proven to be favourable for trematode infections (Selementas & DeWaal, 2015). Ungulate game populations include red deer, roe deer, fallow deer and wild boar. All the mentioned species are potential hosts of *F. magna* and infections were documented in this area in all of them. Therefore, infected red deer and fallow deer are a potential source of infection for wild boar in this area. The wild boar spring fund numbers approximately 300 individuals, with the hunting bag ranging from 150 to 180 wild boars, depending on the season. The roe deer population has decreased dramatically.

Gross and microscopic examination

The livers were examined and observed lesions noted. Each liver was dissected to 2 cm thick slices, which were thoroughly examined for the number of flukes, the presence of migratory channels and pseudocysts, their appearance and maturity, and the presence of other parasites. Samples of liver parenchyma were taken for histological analysis and stored in 10 % buffered formalin. Routine histology using H&E staining was performed. The content of the pseudocysts and faecal samples were analysed by flotation (Zajac & Conboy, 2012), with Sheather's sucrose solution (s.g.1.27).

Results

Parts of the peritoneal cavity and the intestines of the collected wild boars were dark due to the iron-porphirine pigment. Macroscopically, the livers were covered with various amounts of fibrin, and traces of pigment were visible through Glisson's capsule (Fig. 1A). Occasionally, areas where migrating fluke penetrated into the liver were visible. The changes observed on the cut surface of the livers were migratory paths, thick-walled pseudocysts containing two flukes, and degrading pseudocysts. One liver had enlarged bile ducts due to an infection with the liver fluke *Fasciola hepatica*. Live *F. magna* flukes were recovered from six livers and were up to 7 cm long (Fig. 1B). Other pseudocysts contained degrading flukes. An interesting finding was one enlarged pseudocyst filled with fluid content and containing two flukes (Fig. 1C). This pseudocyst had a thin wall, as seen in red deer livers. Only one liver was declared negative.

Histological examination revealed approximately 4 mm thick layers of connective tissue, infiltrated with eosinophils, macrophages and plasma cells. Oval trematode eggs were visible (size 90 – 115 µm) (Fig. 1D). Traces of pigment were present throughout the whole segment. Additionally, the liver infected with both *F. magna* and *F. hepatica* had dilated bile ducts (approx. 1.5 cm in diameter) with thickened walls, containing connective tissue and hyperplastic cubic epithelial cells. Similar to the parenchyma around the pseudocysts, inflammatory cells infiltrated these sections too. Remnants of the parasite (*F. hepatica*) were visible in sections of the bile ducts.

Coprological examination was negative for *F. magna* eggs. Other findings included *Metastrongylus* spp. and *Trichuris suis* eggs, and *Eimeria* spp. oocysts. The pseudocysts that contained live flukes were filled with *F. magna* eggs.

Discussion

In the context of fascioloidosis, wild boars are described as dead end hosts. According to Pybus (2001) and Králová-Hromadová *et al.* (2016) dead end hosts do not contribute to the maintenance and spread of fascioloidosis. Infections in these animals rarely result in the sexual maturation of parasites and production of a few eggs. Finally, it is mentioned that, in this type of hosts, *F. magna* infections may have lethal effect. However, since this group of hosts is rather diverse (including moose, sika and sambar deer, cattle, bison, yak, muskox, collared peccary, llama, etc.) the outcome of infection may differ significantly according to the animal species. In the case of feral pigs (previously domestic pigs, and feral pig x wild boar hybrids) in the USA, Foreyt and Todd (1975) found extensive lesions of their livers, but only up to two flukes per animal. The occurrence of liver lesions was as high as 69 %. On the other hand, Schwartz *et al*. (1993) described findings of pigment traces, inflammatory infiltrate and characteristic operculated eggs of *F. magna* in the liver parenchyma (due to the sectioning of the tissue) of feral pigs from Texas, USA. However, no adult flukes were recovered from the livers. During her PhD work, Giczi (2008) reported findings of black strips (pigment traces) in the liver of wild boar shot during 2005 in Hungary. In the present study, extensive alterations caused by *F. magna* were observed in wild boar livers, including interstitial and prolipherative hepatitis and fibrosis. Chronic prolipherative cholangitis is attributable to

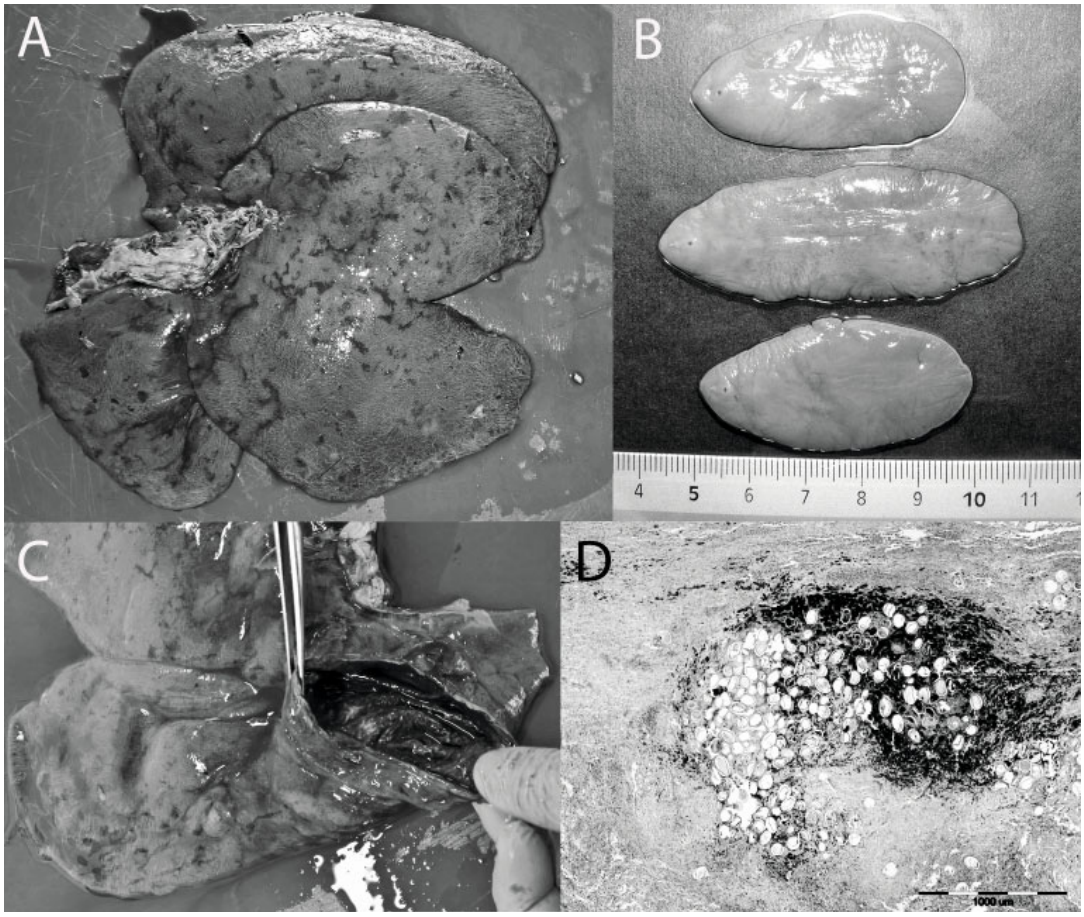

Fig. 1. A – Wild boar liver showing characteristic iron-porphirine traces and fibrinous perihepatitis; B – Mature Fascioloides magna flukes extracted from wild boar livers. Length of flukes varies from 5 to almost 7 cm; C – Atypical large thin-walled pseudocyst filled with fluid content and containing two flukes; D – Section of the liver. Connective tissue with inflammatory infiltrate, pigment and numerous parasite eggs. H&E staining, 4x magnification, scale bar inserted.

F. hepatica infection. It is interesting that, despite the fact that 11 examined livers were infected, only a few intact (live) flukes were recovered. Such findings led us to the conclusion that *F. magna* migratory stages are able to penetrate wild boar livers, but become entrapped within thick-walled pseudocysts. Eventually the flukes die, and only remnants of their cuticula can be found within these pseudocysts. According to the coprological findings, a connection between pseudocysts and bile ducts cannot be established so far. An unusual finding is large thin-walled pseudocysts, which could enable the longer survival of the fluke. Prolonged survival of flukes in combination with less formation of connective tissue could result in establishment of a connection between pseudocysts and bile ducts. In that case, large wild boar populations and their migrating characteristics would enable the even faster spread of fascioloidosis. According to the game managers, the prevalence of positive adult (>2yrs) wild boar in the studied area varies per season, but it can reach approximately 50 %.

It may be concluded that *F. magna* can cause massive and fertile infections in wild boars, but the nature of the pseudocyst formation prevents the long-time survival of flukes and transmission of eggs to the faeces. This course of events currently shows wild boar as a dead end host, with no direct role in the maintenance of fascioloidosis. The possible development of a host-parasite relationship in the future could lead to the establishment of a pseudocyst – bile duct connection.

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