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The occurrence and control of the Big American liver fluke parasitosis in deer in Gemenc

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Abstract

The Big American liver fluke (*Fascioloides magna*) has emerged as a significant parasitic threat to red deer (*Cervus elaphus*) populations in Gemenc, a key natural reserve within the Danube-Drava National Park, Hungary. This research systematically surveyed infections in two hunting areas, Gemenc and Karapancsa, through diagnostic examinations of liver samples collected from hunted deer during and outside the hunting seasons. The study revealed a high prevalence of infection among adult deer, ranging from 73.7% in the 2022/2023 hunting season to 60.3% in the 2023/2024 season, with notable geographic variation. Calves under six months were initially free of infection, but older calves showed increasing susceptibility in the second year, highlighting growing transmission risks. No significant differences in infection rates were observed between sexes. The study revealed variations in infection intensity and severity across hunting areas. The findings highlight the need for integrated management strategies, including targeted anthelmintic interventions (e.g., triclabendazole, albendazole) and habitat monitoring to disrupt the parasite's life cycle.

Összefoglaló

A nagy amerikai májmétely (*Fascioloides magna*) jelentős állat-egészségügyi kockázatot jelent a gímszarvas (*Cervus elaphus*) populációra Gemencen, a Duna-Dráva Nemzeti Park egyik legfontosabb természetvédelmi területén. Kutatásunkban két vadászterületen, Gemencen és Karapancsán mértük fel a nagy amerikai májmétely fertőzöttséget a vadászidény alatt és azon kívül elejtett gímszarvasokból gyűjtött májminták diagnosztikai vizsgálatával. Kutatásunk a tenyészérett szarvasok körében a nagy amerikai májmétely magas előfordulási arányát mutatta ki, amely a 2022/2023-as vadászati szezonban átlagosan 73,7%-os és a 2023/2024-es szezonban 60,3%-os volt, jelentős földrajzi eltérésekkel. A megvizsgált hat hónaposnál fiatalabb borjak még mentesek voltak a fertőzéstől, de az idősebb borjak a második vadászati szezonban már kismértékű fertőzöttséget mutattak, ami rávilágít a növekvő fertőzési kockázatra. Az ivarok között nem találtunk jelentős különbséget a fertőzési arányokban. A kutatásunk a fertőzés intenzitásának és súlyosságának eltéréseit is kimutatta a Gemencen belüli egyes vadászterületek között. Az eredményeink rávilágítanak az integrált állat-egészségügyi protokollok fontosságára Gemencen, beleértve a nagy amerikai májmétely elleni célzott antiparazita kezeléseket (pl. triklabendazol, albendazol) és az élőhely folyamatos monitoringját a parazita életciklusának megszakítása érdekében.

Contents

1. Introduction

Fascioloides magna, commonly known as the giant liver fluke, poses a considerable risk to cervid populations worldwide. In Gemenc, a prominent natural reserve and part of the Danube-Drava National Park in Hungary, the presence of flukes in deer has been a matter of concern among conservationists and wildlife researchers. The life cycle of *F. magna* includes complex relationships between definitive hosts, such as deer species, and intermediate hosts, including freshwater snails. To effectively control the effects of the parasitosis on deer populations, management plans must take these dynamics into account.

Gemenc's unique ecological characteristics, including its diverse habitats and rich biodiversity, create an ideal environment for the giant liver fluke, and recent observations indicate a trend of increasing fluke infections among deer, prompting the necessity for wide-ranging research and intervention strategies. Additionally, the forest's value as a recreational area and its impact on biodiversity conservation highlight the need for comprehensive research on the health of its wildlife population.

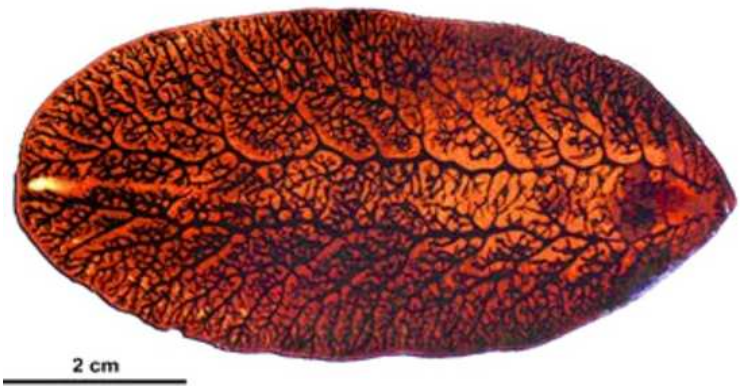
This thesis aims to give a comprehensive examination of the prevalence, distribution, and impact of *Fascioloides* infection in deer within the Gemenc forest ecosystem, including spatial distribution patterns and potential factors influencing transmission dynamics. Additionally, we aim to inform evidence-based management decisions and conservation strategies to preserve the health and sustainability of the habitat. By analysing data, based on a combination of field surveys, laboratory, and pathological analyses, we request to clarify the factors contributing to the rise in fluke infections and identify potential intervention points to alleviate their spread.

2. Literature review

2.1. Taxonomy and morphology of the Large American Liver Fluke

The *Fascioloides magna* finds its taxonomic belonging within the flatworms, Platyhelminths, in the class of the Trematoda, the so-called flukes [1, 2]. The parasite measures 70-100 mm length and 30-40 mm width and is therefore the biggest representative of the liver flukes and thus the term large American liver fluke is commonly used [3] (**Figure 1**).
Morphologically all flatworms are dorsoventrally flattened and not segmented, showing a bilaterally symmetry, without a body cavity, a respiratory or a circulatory system [1, 4]. A unique connective tissue forms its parenchyma that embeds the intestinal canal, which emerges from branched caeca, which are attached to the oral sucker through the pharynx. *F. magna* is hermaphrodite, the parasite possesses fully developed genitalia of the male and the female gender [5]. The male reproductive system consists of two testes leading to the vas deferens and finally in the cirrus (penis), enclosed in a sac. The female genitals own a single ovary with oviduct and a uterus, containing many eggs, filling up the majority of the body cavity within the convoluted uterus. Testes and uterus find their ending together in the genital opening. Despite the oral sucker, the worms have another muscular sucker, located ventrally, both suckers are used for attachment. Metabolic processes are done by the tegument which builds up the leaf-like body surface [1, 4].

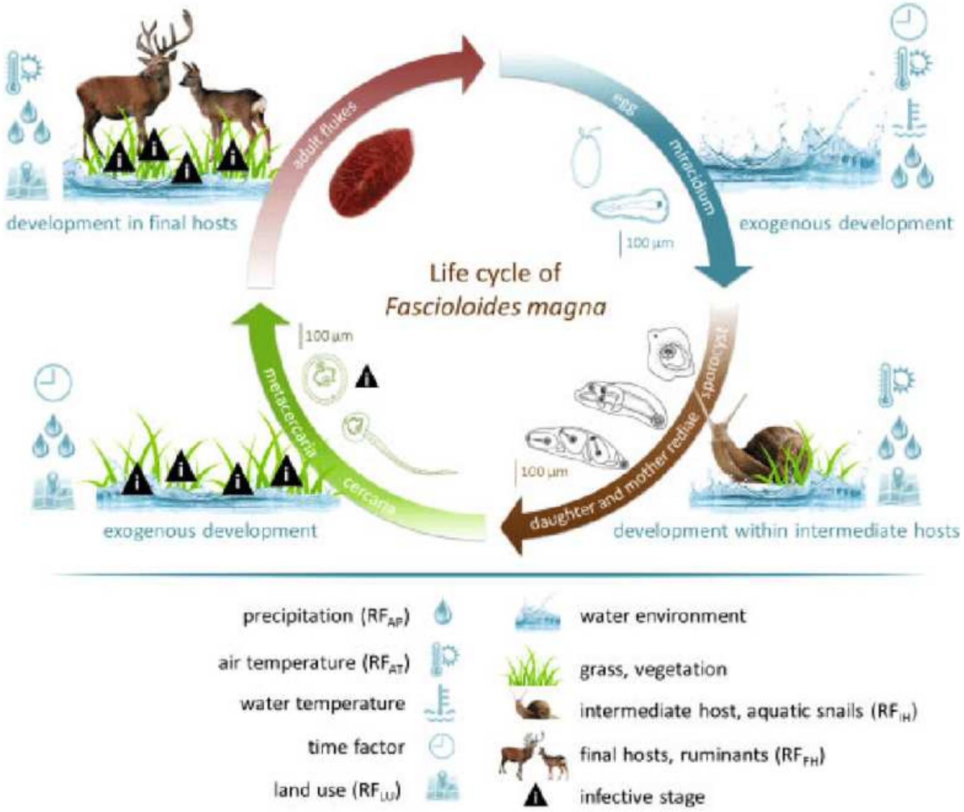
Figure 1. Adult stage of *Fascioloides magna* [2]



2.2. The life cycle

The life cycle of the family *Fasciolidae* is indirect, needs an intermediate host, and consists of five larval stages: the miracidium, the sporocyst, the redia, the cercaria stage and the infective metacercaria stage [1, 6] (**Figure 2**).

Figure 2. The life cycle and developmental stages of *Fascioloides magna* [7]



Sexually active flukes living in the biliary ducts of their hosts, which pass the worm eggs through their faeces to the environment. The *Fascioloides* egg measures 110 to 160 µm, containing the zygote, are yellowish in colour and have an operculum. In a proper external environment with temperatures ranging from 15°C and 30°C, adequate oxygen, and aquatic environment, the eggs develop into ciliated larvae, the so-called miracidia [3]. After four to seven weeks the miracidium actively swims for their intermediate hosts, aquatic snails. Due to an eye spot which is phototactic and their attraction to chemicals produced by the lymnaeid snails, they can easily detect and infect them. Inside the intermediate host the asexual multiplication takes place and sporocysts develop in the snail's pulmonary sac. The replication is asexual (parthenogenesis) in case of sporocysts and rediae stages, the sporocysts produce rediae which produce daughter rediae within themselves [1, 3]. The daughter stages develop into cercariae which emerge from the snail host and then encyst in the environment becoming the infective metacercariae [2]. This last larval stage remains viable for a prolonged time in suitable conditions and the transmission to a final host happens during two major periods: in spring and late summer [6]. During this time, the development inside the intermediate hosts lasts for six to nine weeks and depends on environmental factors as temperature as well. The ungulates, the final hosts, get infected while grazing by the metacercaria or accidentally by an infected intermediate host. The intestinal wall of the final host gets penetrated by the juvenile fluke which migrates to the liver, after four to seven weeks the parasite enters the bile ducts where it becomes mature. In the liver parenchyma the mature can be found in pseudofibrosis cysts where the parasite feeds on blood of its host and shed its eggs [1].

2.3. Spectrum of Definitive hosts

2.3.1. The primary definitive hosts

Final or definitive hosts are those in which the *Fascioloides magna* successfully matures in the liver, their eggs are passed to the gastrointestinal tract via the biliary ducts and patent infections are recognised. Therefore, in enzootic environments, only definitive hosts offer favourable conditions for supporting a fluke population [6]. The natural host spectrum of *F. magna* is the white-tailed deer (*Odocoileus virginianus)* [2]. However, the range is much broader including domestic and free-living ruminants [6]. In North America, the definitive host group includes white-tailed deer (*O. virginianus*), wapiti (*Cervus canadensis*), caribou (*Rangifer tarandus*), and black-tailed deer (*O. hemionus hemionus*), whereas in Europe, it includes red deer (*C. elaphus*) and fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*), all are tolerant to infection and where the endogenous phase of the development cycle runs through a typical course [2, 8]. Besides there were also mentioned some experimental infections in other species, like sheep, goats, llama, rabbits, and guinea pigs [1].

2.3.2. Aberrant hosts

It was proven that although many species are susceptible to infection, in North America as well as in Europe, only the minority has a significant impact to sustain the population of the fluke, because in so-called aberrant hosts the parasite cannot successfully complete their migration and therefore no encapsulation in the liver parenchyma takes place. In addition, immature flukes can enter numerous abdominal and pleural organs, causing haemorrhagic cavitation, interlobular edema, or fibrotic septae. The acute tissue damage caused by migrating immature flukes often has fatal consequences for aberrant hosts, predominately ovine species, such as domestic sheep and goat, mouflon, or roe deer. Very rarely adult flukes as well as the eggs can be found [2, 6].

2.3.3. Dead-end hosts

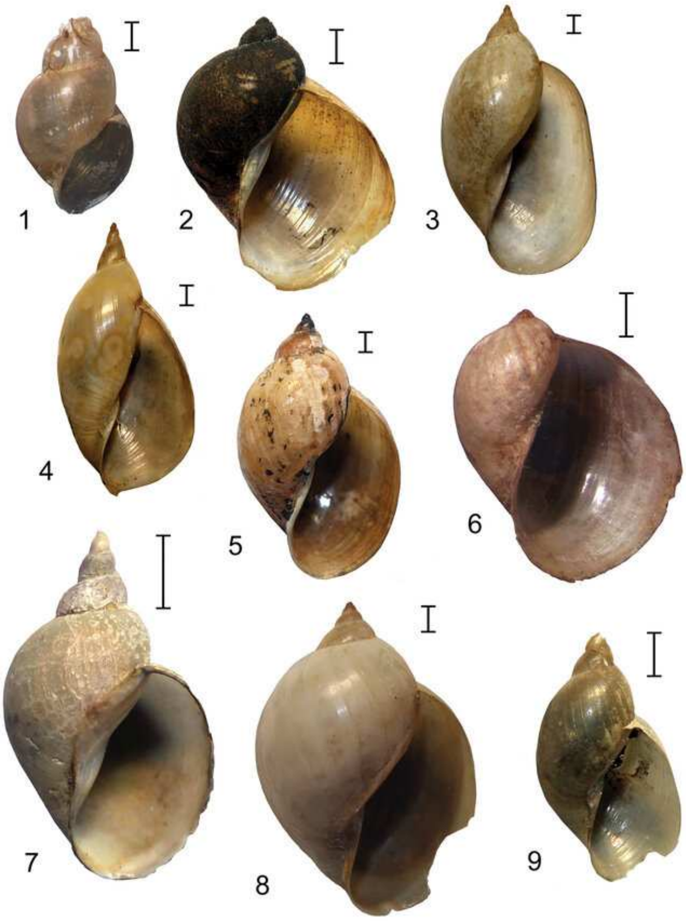
When immature stages successfully enter the host's liver and encapsulate inside the hepatic parenchyma, but seldom mature and only a few eggs are lost, these hosts are referred to be dead-end hosts, such as sika deer, cattle, bison, or domestic pigs [6]. The immune system of dead-end hosts likewise produces a pseudocyst in the hepatic parenchyma, but these concentrate immature eggs because they lack an exit into the environment [2].

2.3.4. Intermediate hosts

Intermediate hosts of *F. magna* are as essential for the parasite as the definitive host. Any interruption in the transmission chain to these two host types will invariably prohibit the fluke from finishing its life cycle. The giant liver fluke has now been discovered in both North America and Europe. Aquatic snails from the genus Lymnaea (subfamily *Lymnaeinae*; family *Lymnaeidae*) have been recognized as appropriate intermediate hosts for *F. magna* in North America [2].

In Europe, the genus *Galba* (syn. Lymnaea) belongs to the same family *Lymnaeidae* and subfamily Lymnaeinae. Individuals measuring 5-11 mm in height including the shell and 3-7 mm in width and are hermaphrodites [2, 3]. They prefer standing waters, and the highest densities were found during the winter months, and they are capable for overwintering, which plays a key role in the life cycle of the large American liver fluke [2]. For example, overwintered fluke eggs inside the intermediate host develop much slower but they are alive if the temperature range is not below the freezing point. Temperature, seasonal conditions, and moisture are crucial factors for the survival of the snails [6]. According to different geographical locations and the according temperature snails show varied susceptibility to miracidia infection. The larval stage of the fluke can penetrate the snail more easily in warm than in cold, deep waters, moreover North American individuals of Lymnaeidae are more resistant to liver fluke infections [2] (**Figure 3**).

Figure 3. Annotated type catalogue of lymnaeid snails (Mollusca, Gastropoda) [9]



Type specimens of Lymnaeidae (ZMB). 1 – Limnaea alfredi Suter, 1890, a paralectotype. 2 – Limnaea ovata var. amnicola Westerlund, 1890, a syntype. 3, 4 – Limnaeus amygdalum Troschel, 1837, two syntypes. 5 – Limnaea javanica var. angustior von Martens, 1881, a syntype. 6 – Limnaea brevispira von Martens, 1897, the holotype. 7 – Limnaea stagnalis var. baltica Lindström, 1869, a syntype. 8 – Limnaeus cerasum Troschel, 1837, a syntype. 9 – Limnaea columella var. championi von Martens, 1899, a syntype. Scale bars: 1 mm (1), 2 mm (2–6, 8–9), 5 mm (7).

2.3.5. Occurrence of intermediate hosts

Five snail species are naturally infected by the large American liver fluke in North America, its native country. These include *Lymnaea modicella, L. caperata, L. bulimoides, L. parva, and L. palustris nuttalliana*. All of them are distributed all over the country, to Panama. The intermediate host has a larger spectrum here because the fluke is native to the country and has therefore had time to adapt [2].

In Europe, as an enzootic country, only two infected snails' species have been established, Galba truncatula and Radix peregra. There are three main locations for *F. magna* in Europe: in

Italy, the natural park La Mandira, in southern and central Bohemia, and in the region of the Danube floodplain forest [2].

2.4. Clinical signs and pathology

Primary lesions in Fascioloidiasis show up as encapsulated flukes in the liver of deer and cattle and are less pathogenic. Affected livers are commonly enlarged with rounded margins and with a fibrinous serosa. As the flukes containing an iron porphyrin pigment, due to blood feeding, some black staining might be visible [6]. The larvae constantly migrate in the tissues causing traumatic hepatitis which may lead to death due to vascular lesions such as thrombosis and endophlebitis in sheep and goat. Within six months affected sheep die. Clinical signs such as lethargy, depression, anorexia, and weight loss occur in aberrant hosts, shortly before they die [6]. Because to the limited migration of juvenile flukes, infection may still be in a subclinical stage in the final hosts; but, with severe infections, lethal infections may occur. However, it might cause mortality in dead-end hosts [2]. The prepatent period is minimum six to twelve weeks [1].

2.5. Diagnosis

The final diagnosis of the parasitosis in final hosts is usually made postmortem. In alive individuals the detection of fluke eggs in faeces is evidence for an infection. Using Sheather's sugar solution for a flotation test let the fluke eggs float due to their high specific gravity.
In dead-end and aberrant hosts only, necropsy gives a reliable diagnosis for an *F. magna* infection, which is based on the severe tissue damage to the migrating larvae and larvae themselves [6]. Adult flukes and their eggs are rarely found in dead-end and aberrant hosts [2]. In the intermediate snail hosts the diagnosis is based on the detection of cercariae from captive or crushed snails. Recently it is not possible to differentiate *Fasciola hepatica* from *Fascioloides magna* using reverse transcriptase-polymerase chain reaction [6].

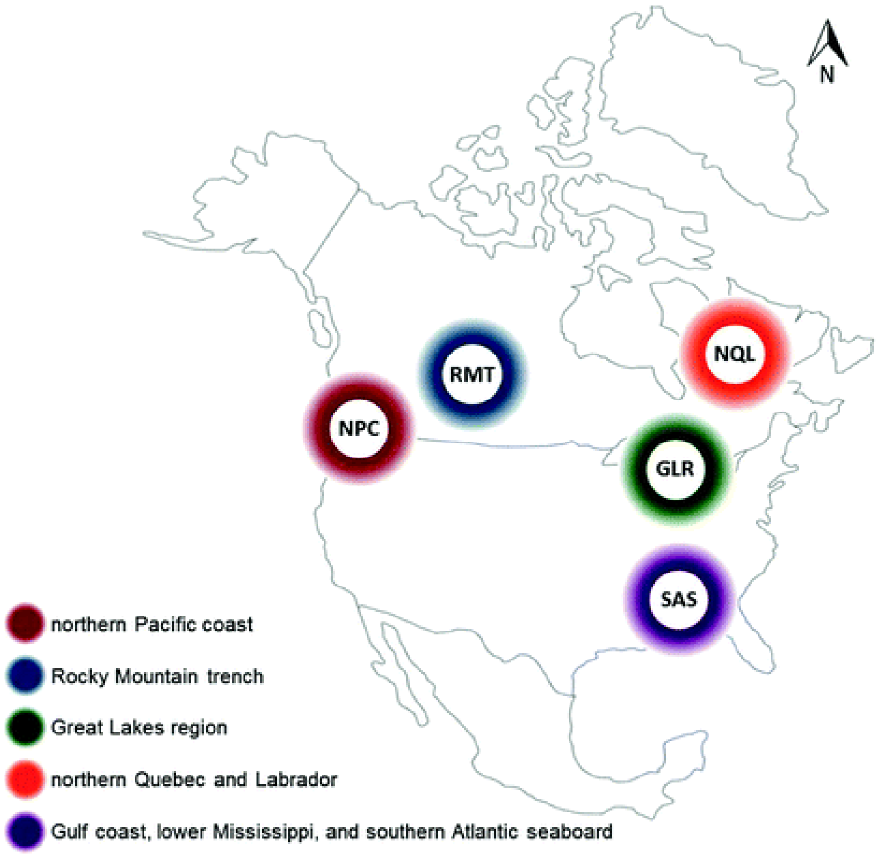
2.6. Geographical prevalence

2.6.1. Native country

Fascioloides magna is native to North America and may be found across the United States and southern sections of Canada, however it is most prevalent in the north-eastern United States and areas bordering the Great Lakes [2]. *F. magna* has been found in five enzootic regions in the United States and southern Canada to date: the northern Pacific coast (NPC); the Rocky Mountain trench (RMT); the Great Lakes region (GLR); northern Quebec and Labrador (NQL);

and the Gulf coast, lower Mississippi, and southern Atlantic seaboard. In Canadian provinces and the US states natural infections of free-living and domestic ruminants were confirmed [6, 10] (**Figure 4**).

Figure 4. US states and Canadian provinces with confirmed natural infections of *F. magna* in free-living and domestic ruminants [10]

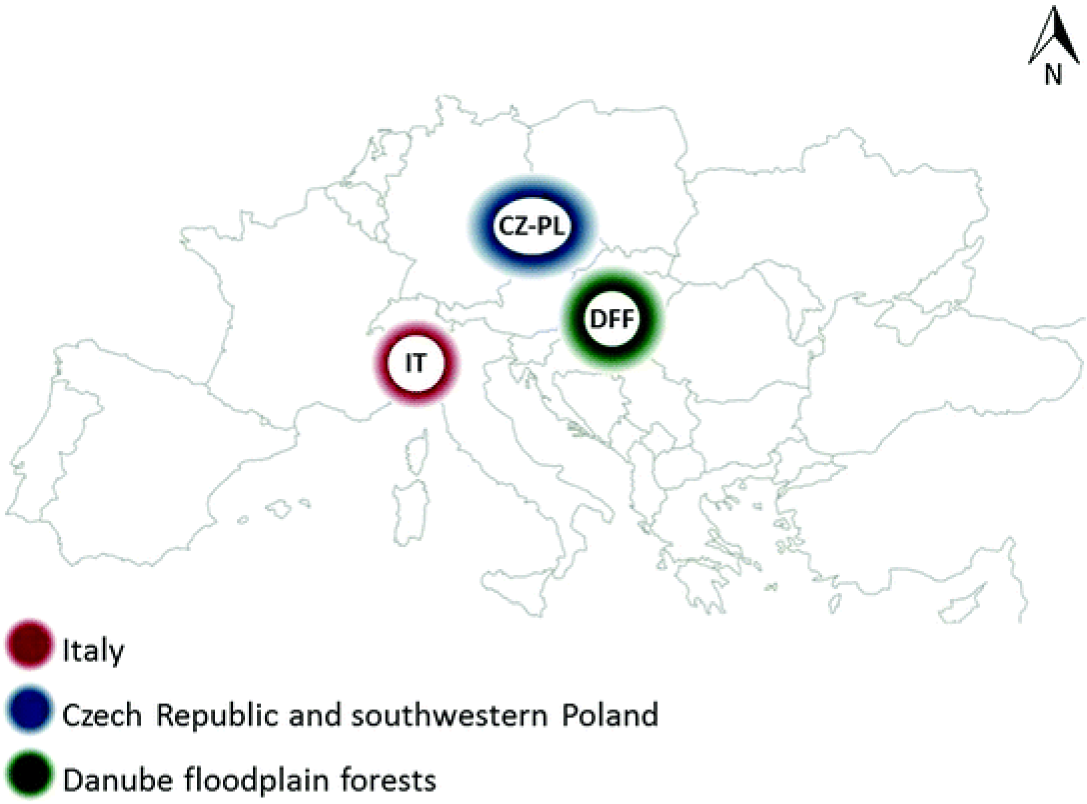


Natural migration and human-directed cervid translocations have altered the distribution of *F. magna* throughout North America. Definitive hosts, such as white-tailed deer, wapiti, and caribou, have had a significant role in the persistence of fascioloidiasis in the natural environment and its dissemination into currently identified enzootic zones [6, 10]. The population of white-tailed deer dropped significantly during the 16th and 20th centuries, moving from the east to the west of North America, resulting in the extinction of the gigantic liver fluke over most of its previous habitat. Nonetheless, fluke populations may have maintained in three important refuge locations, namely in the RMT region with persisting wapiti populations, the SAS region with ongoing prevalence of white-tailed deer, and the NQL region [10].

2.6.2. Enzootic regions

Fascioloides magna was established into Europe with its imported deer hosts in the nineteenth century for a variety of purposes, including hunting, aesthetics, and an attempt to improve the heads of native red deer [11]. Three permanent natural foci established: La Mandria Regional Park in the northern Italy (IT); Czech Republic and southwestern Poland (CZ-PL), and Danube floodplain forests (DFF) [10] (**Figure 5**).

Figure 5. Schematic of European natural foci of F. magna [10]



The first reports of *F. magna* in Europe came from Italy, 1865 originally discovered by Bassi, (1875), in the fenced-in "La Mandria" park [10]. Later the fluke was found in former Germany, Lower Silesia, and former Czechoslovakia. In Italy, red deer, wapiti, fallow deer, and other exotic and domestic ungulates were therefore exposed to the fluke, as were wild red deer in Germany [11].

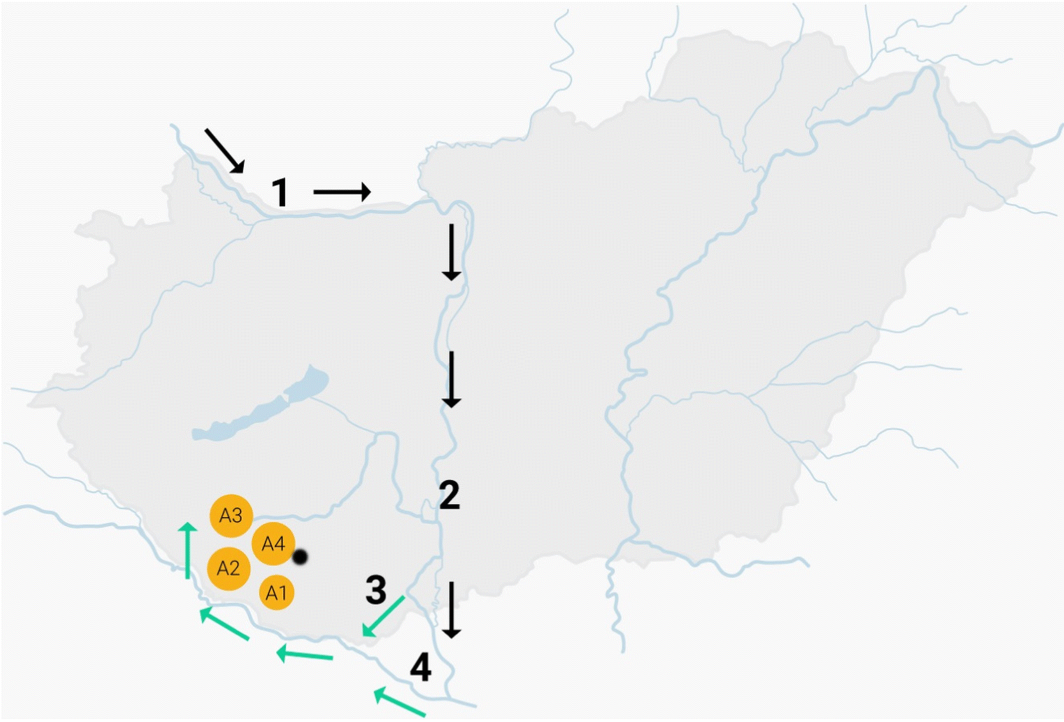
Within Europe the Italian regional park represents first stable and isolated natural focus of Fascioloidiasis with infections primarily in red deer, but also a broad range of dead-end hosts, mainly cattle, horse and wild boars, as aberrant hosts, like sheep and goat are determined [10]. For the first time in former Czechoslovakia, a fallow deer was found in a Moravian game preserve. White-tailed deer and wapiti, on the other hand, have been brought into and translocated between Moravia and Bohemia [11]. Through this the second European focus of

Fascioloidiasis was determined in 1910. *F. magna* was discovered in definitive hosts such as red deer, fallow deer, or white-tailed deer, as well as sika deer and cattle (dead hosts), and roe deer (aberrant host) [10].

Later, the fluke was described in several additional European nations where red deer, fallow deer, and introduced white-tailed deer are the definitive hosts. *F. magna* reports from Spain and the Polish-German border were scattered [8].

The third European focus is Danube floodplain forests, encompassing Austria, Slovakia, Hungary, and Croatia where the first finding of *F. magna* was documented in fallow deer in Lower Austria in 1982 [10, 11]. As no permanent focus established this infection can be considered as a sporadic finding [10]. The real outbreak of Fascioloidiasis in the Danube region was documented in 1988 in a red deer in south-western Slovakia [10, 11]. Shortly after infections were found across the Slovak-Hungarian border, in the north-western part of Hungary known as Szigetköz [11]. The parasite was observed especially in Austria and Croatia and additionally in other territories along the Danube River, like eastern Croatia and Serbia [10]. The origin of *F. magna* in the Czech Republic and the Danube region is uncertain, as there is no indication of game import from North America or enzootic places in Europe [8]. *F. magna* has been found in the Danube floodplain woodlands on a regular and recurring basis since its discovery in the various nations and representing the red deer as the most frequent and dominant definitive host. Furthermore, Fascioloidiasis was seen in fallow and roe deer. Thus far, the Danube floodplain woods are Europe's sole natural centre with no known *F. magna* infection in domestic ruminants [10] (**Figure 6**).

Figure 6. Presumed spreading of *F. magna* [12]



Note: Black numbers and arrows show the spreading in Hungarian passage of Danube floodplain and in Croatia. A1, A2, A3, and A4 were the screened hunting estates in the study and the green arrows show the presumed spreading of *F. magna* in the floodplain of River Drava. The black dot shows are deer enclosure where *F. magna* has appeared in 2015.

2.7. Treatment and prevention

For therapeutical treatment triclabendazole, albendazole, clorsulon and closantel are used in cattle and sheep at 20 mg/kg orally, for deer triclabendazole 2 x 10 mg/kg on two consecutive days is used. Drug combinations are proven to have higher efficacy [1]. Besides treatment prevention plays a significant role, therefore separation of livestock and cervids, elimination of cervid definitive hosts, or fencing of cattle away from natural wetlands in enzootic areas were all priorities. Furthermore, a variety of physical (i.e., drainage) or chemical (copper sulphate) wetlands treatments were promoted in attempt to minimize or destroy snail populations. Unfortunately, most of the wetland treatments have a significant environmental impact [6].

3. Material and Methods

The aim of the study is to develop a strategy for the control of red deer population against the American large liver fluke infection in the hunting area of Gemenc Inc. As a first step, it is to survey the prevalence of American liver fluke infection in the two relatively well-defined hunting areas, Gemenc and Karapancsa, by doing diagnostic examination of the livers from shot red deer both during and out of the hunting season. In Gemenc, the estimated red deer population for the 2022/2023 hunting season was 1330 individuals, with a planned hunting of 345 individuals during the hunting season. In Karapancsa, the estimated red deer population was 305 individuals, with a planned hunting of 92 individuals.

To estimate the extent of American liver fluke infection within the red deer population and determine the initial stages of the parasites, diagnostic examination of every suspicious-looking liver from the individuals shot during the hunting season is required. The condition is that at least 40 livers infected with these flukes, which have not been frozen, are needed annually from Gemenc, and at least 10 from Karapancsa. Additionally, for estimating an infection within the population, random diagnostic examinations of seemingly infection-free livers are also required. The determination of the number of these examinations is entrusted to the hunters of Gemenc Inc., but we consider a maximum of 40 livers from Gemenc and 10 from Karapancsa, appearing healthy, to be adequate and will be performed annually as part of this research.

Furthermore, to examine the viability of American liver fluke eggs, diagnostic examination of every suspicious, unhealthy liver is necessary for any red deer shot outside the hunting season for any reason. Based on the diagnostic examinations of the livers, we will estimate the extent of infection, which will serve as a positive control for assessing the effectiveness of antiparasitic treatments. Subsequently, based on the results of diagnostic examinations, the proposed defence strategy will be developed and, if necessary, modified and refined, taking the regional infection data into account. The diagnostic examinations on the deer livers and fluke eggs are done by the University of Veterinary Medicine in Budapest.

During the examination process a related new methodology was developed to characterize the progression of *F. magna* infection and describe the functional stages of liver fluke infection. According to this, the infection stages characterized by distinguishable pathological changes are as follows: acute infection, subacute infection, and chronic infection. Although characteristic pathological changes may be present mixed in the livers of individual hosts, from a practical standpoint, individuals are always classified into categories considering the potential egg shedding.

"Acute infection" refers to the stage of migrating flukes following a new infection and liver damage, during which developing flukes and thus infected hosts don’t shed infective eggs into the environment (so-called prepatent period). Unfortunately, some flukes may already shed eggs before completing migration, so this stage is more significant in terms of monitoring the dynamics of infection, while it is worth considering subacute stage regarding egg shedding.
The characteristics of "subacute infection" include typical encapsulated fluke cysts, mature flukes, and active shedding of infective eggs (the so-called patent period). In the "chronic infection" stage, the flukes have died and been absorbed, but some of the eggs in contracted cysts may still be infective, although they can only be released into the environment after the death of the animal, with the intestines. The identification and quantification of these pathological or fluke developmental stages are necessary for estimating the current and expected dynamics of the infection.

4. Results

In the first year (01.09.2022-31.08.2023) of the research, instead of the planned 100 samples, 125 liver samples were received for detailed parasitological and post-mortem examination. The origin, infection status, as well as the distribution by age and sex of the liver samples deviated to some extent from the planned structure in the final sample (**Table 1**).

Table 1. Characteristics of processed liver samples between 01.09.2022-31.08.2023

Hunting area	Livers showing clinical signs						Apparently infection-free livers					
	stag		doe		calf		stag		doe		calf	
	plan	fact	plan	fact	plan	fact	plan	fact	plan	fact	plan	fact
Bátaszék	1	1	2	2	1	1	1	1	1	1	1	1
Szekszárd	8	9	8	1	4	1	8	13	8	10	4	33
Hajós	3	3	3	3	2	0	3	3	3	2	1	1
Pandúr	4	6	3	8	2	2	4	2	3	2	2	6
Karapancsa	4	4	4	3	2	0	4	1	4	1	2	4
Total	**20**	**23**	**20**	**17**	**11**	**4**	**20**	**20**	**19**	**16**	**10**	**45**

Note: the design numbers with a green background have been fulfilled.

In the second year (01.09.2023-31.08.2024), instead of the planned 100 samples, 109 liver samples arrived for detailed parasitological and pathological examination (**Table 2**).

Table 2. Characteristics of processed liver samples between 01.09.2023-31.08.2024

Hunting area	Liver showing clinical signs						Apparently infection-free livers					
	stag		doe		calf		stag		doe		calf	
	plan	fact	plan	fact	plan	fact	plan	fact	plan	fact	plan	fact
Bátaszék	1	1	2	7	1	1	1	2	1	7	1	5
Szekszárd	8	8	8	0	4	0	8	1	8	2	4	33
Hajós	3	1	3	3	2	2	3	1	3	3	1	3
Pandúr	4	7	3	10	2	0	4	4	3	3	2	13
Karapancsa	4	2	4	3	2	0	4	1	4	2	2	2
All together	**20**	**19**	**20**	**23**	**11**	**3**	**20**	**9**	**19**	**17**	**10**	**38**

Note: the design numbers with a green background have been fulfilled.

According to field evaluations, 81 livers were classified as healthy and 44 as infected. However, after pathological and parasitological examinations, these classifications were revised to 63 true negatives and 62 true positives for *Fascioloides magna* infection. These results suggest that field assessments of liver condition may significantly underestimate the actual prevalence of liver fluke infections.

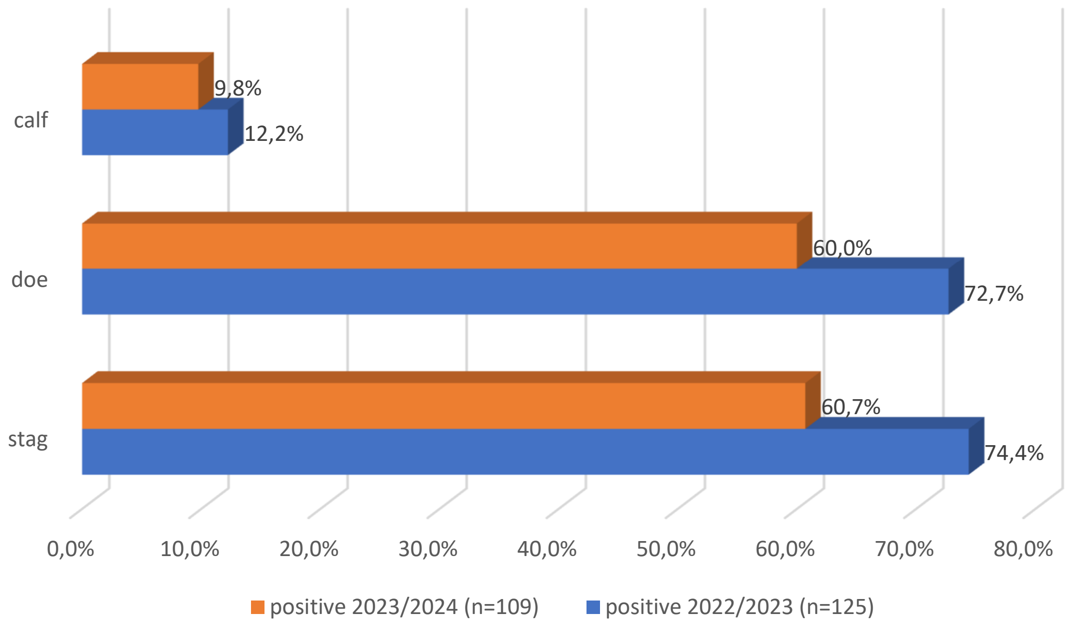
In the second year 64 healthy and 45 infected liver samples were examined. Based on pathological and parasitological examination, these values changed to 65 true negatives and 44 true positives, indicating *Fascioloides magna*-infected liver samples. This result is very good compared to the first year's underestimated infection rate (**Table 3**).

Table 3. Classification of liver samples submitted and processed

	Submit rating	pcs	Test qualification	pcs	%
2022/2023 (n=125)	Apparently “infected”	44	true positive	62	49.6%
	Apparently “healthy”	81	true negative	63	50.4%
2023/2024 (n=109)	Apparently “infected”	45	true positive	44	40.4%
	Apparently “healthy”	64	true negative	65	59.6%

As partially expected, the proportion of adult red deer is significantly higher in showing infection as calves up to one year of age. There was no significant difference between the degrees of infection between the sexes in both years of the study. Overall, both does and stags were equally infection-free, with slightly less than 25% being free of infection, which is a better ratio than the previous year's value (**Figure 7**).

Figure 7. ***F. magna*** **infection according to age and sex**



Only around a quarter of the does and stags were completely free of infection, with the majority of animals having a degree of fluvial illness diagnosed. In both examination periods there was no apparent correlation between the specific age of the examined adult individuals and the rate of infection (**Figure 8-9)**.

Figure 8. ***F. magna*** **infection by age in 2022/2023 (n=125)**

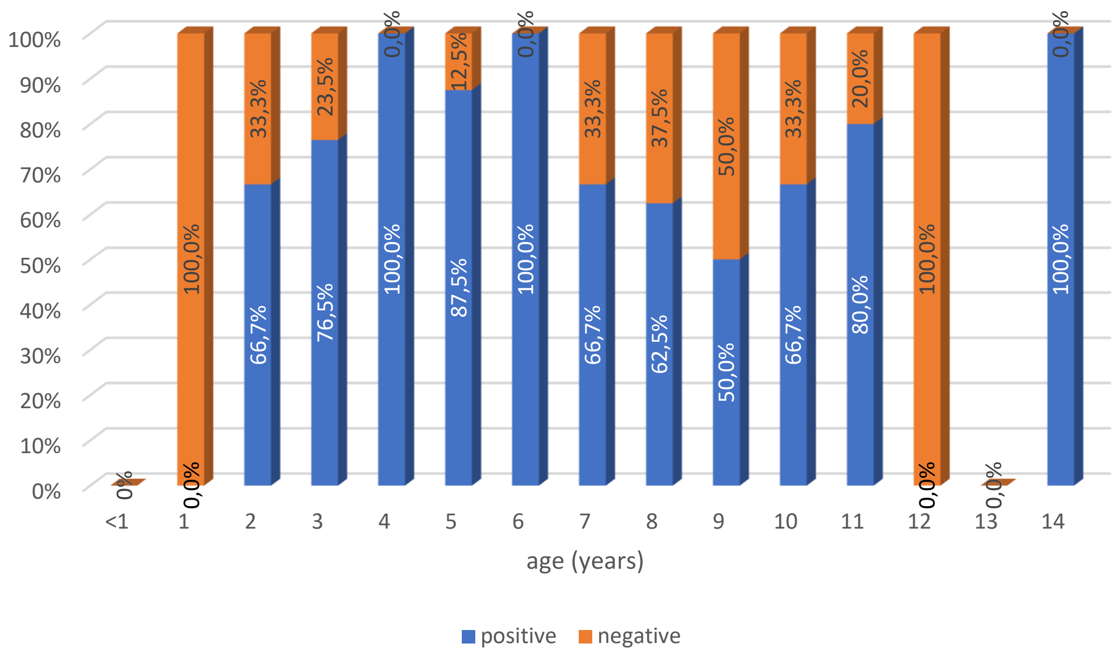
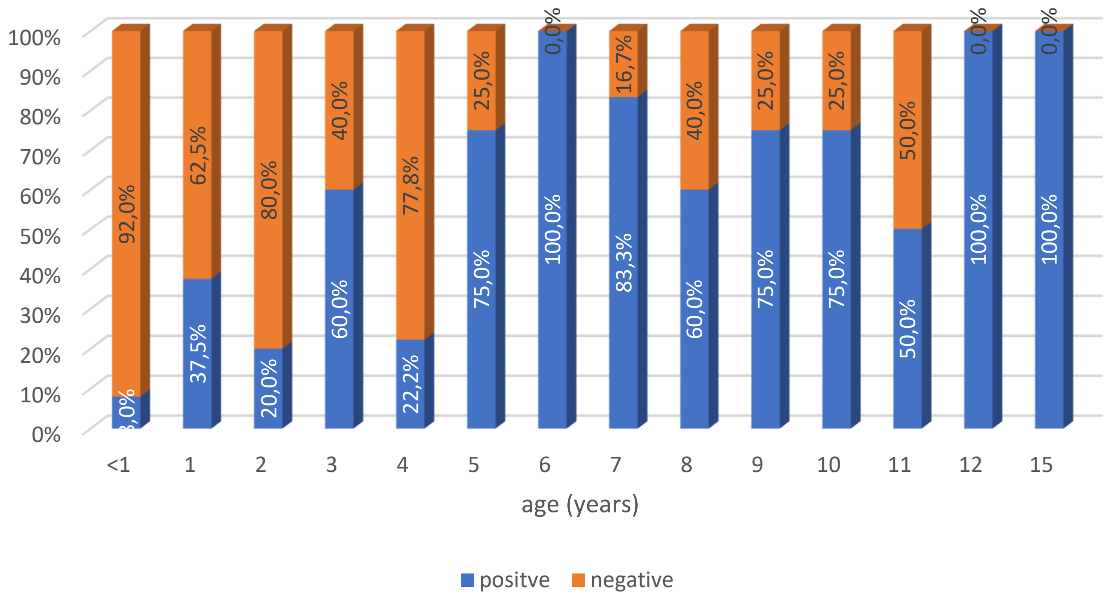
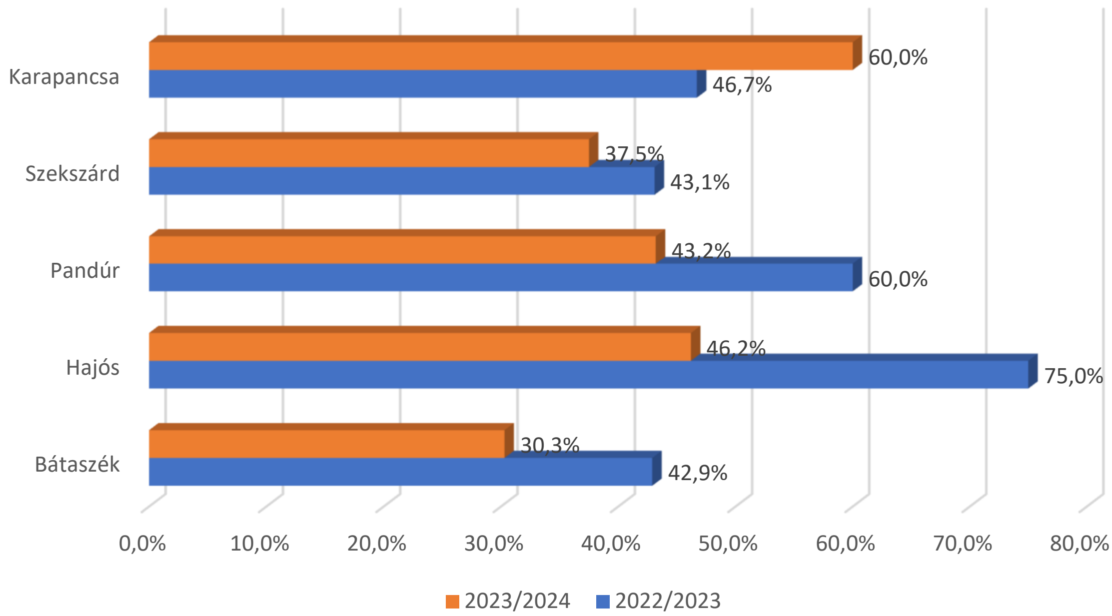


Figure 9. ***F. magna*** **infection by age in 2023/2024 (n=109)**



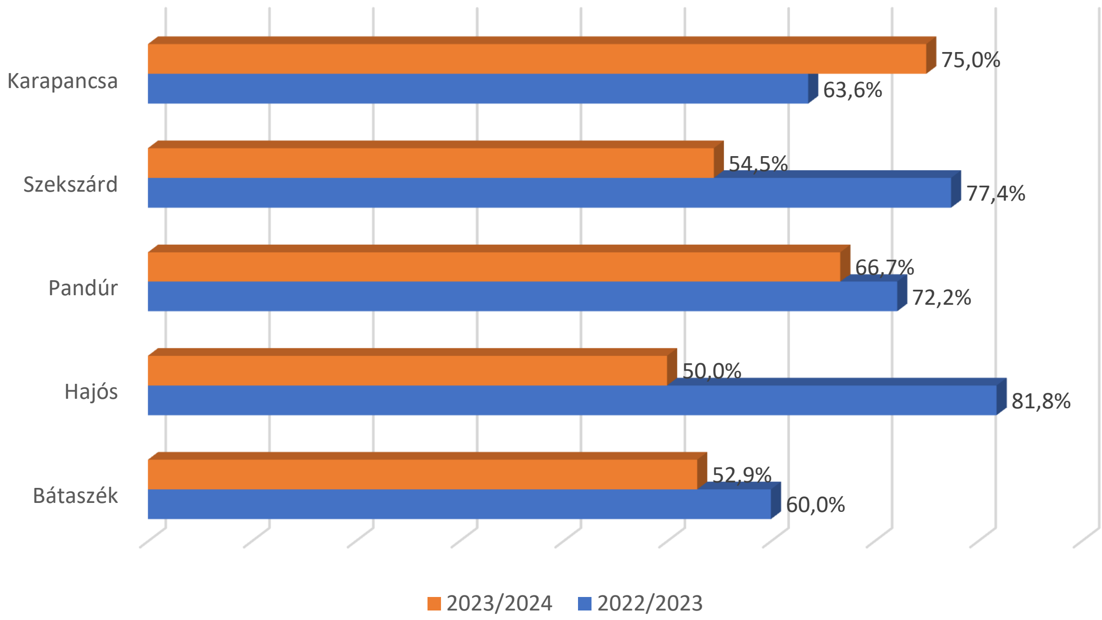
In 2023/2024 the overall prevalence of infection for the entire red deer population broken down by forest areas examined by Gemenc Forestry Company is 40.4% (compared to 50% during the 2022/23 period) (**Figure 10**).

Figure 10. Change in the prevalence of infection in the different hunting seasons by areas



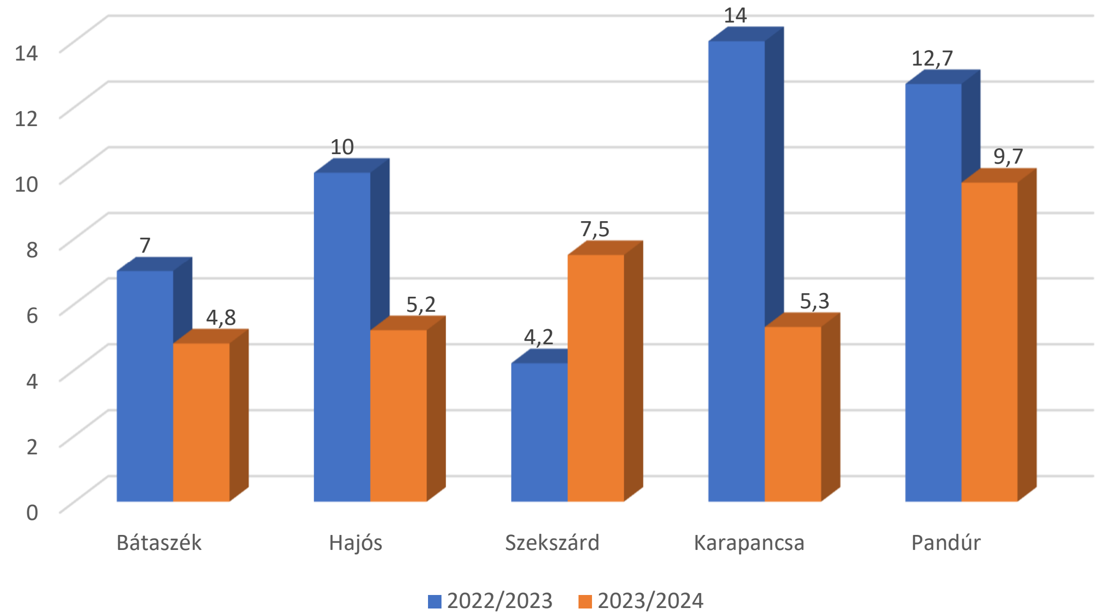
The prevalence rates calculated only for adult individuals showed significantly lower infection rates in 2023/2024, with 60.3% for the entire examined red deer population in Gemenc Inc. (compared to 73.7% one year earlier). Broken down by hunting areas, the prevalence rate was the highest in Hajós in the first year of the study and lowest in Bátaszék, in the second year Hajós showed lowest and Karapancsa the highest infection rates (**Figure 11**).

Figure 11. Prevalence of infection only in adult individuals in comparison



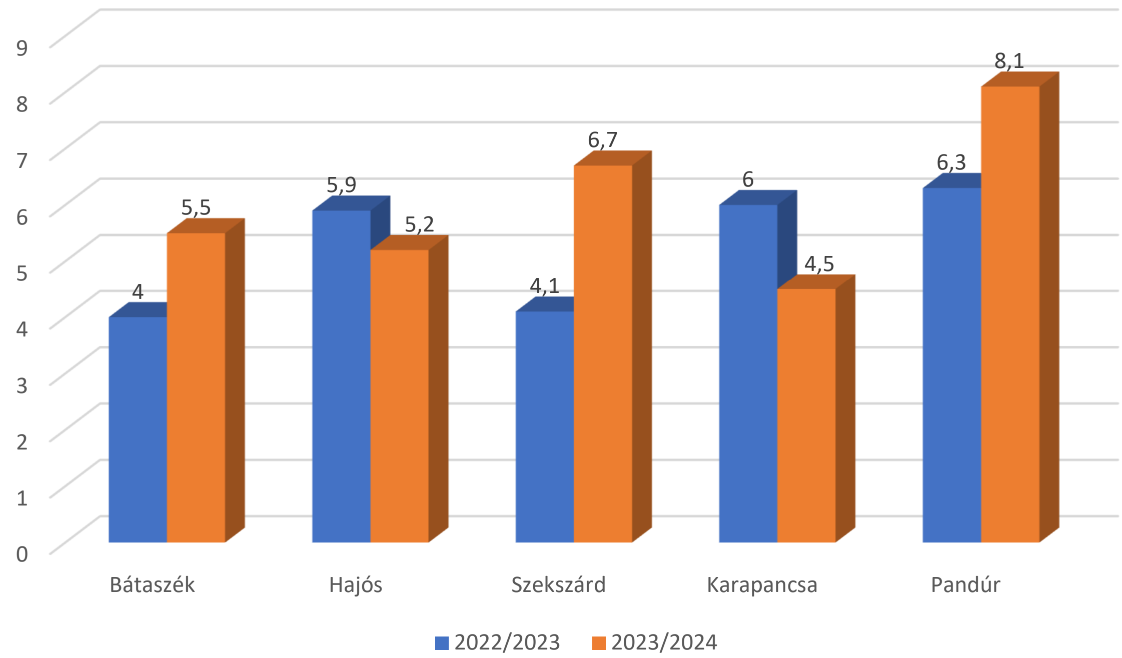
Among the general epidemiological indicators, the intensity of the fluke infestation (number of flukes in infected individuals) varied between 4.2 (Szekszárd) and 14 (Karapancsa) in 2022/2023. In the 2023/2024 the intensity ranged from 4.8 (Bátaszék) to 9.7 (Pandúr) **(Figure 12)**.

Figure 12. Change in intensity of liver fluke infections



To characterise the severity of infection, we used the number of lesions found in infected livers (foci). The severity of liver fluke infection ranged from 4 (Bátaszék) to 6.3 (Pandúr) in 2022/2023 and from 4.5 (Karapancsa) to 8.1 (Pandúr) in 2023/2024. Overall, the severity of infection did not change, but from the data, it can be seen that only the Karapancsa area saw an increase in the proportion of infected samples, while in the other hunting areas, it decreased (**Figure 13).**

Figure 13. Change in severity of liver fluke infections



In the stage of acute infection, the fewest individuals were found in both hunting years of the study, and there were areas (Bátaszék, Hajós, Karapancsa) where it did not occur at all in the first hunting year. This may indicate the absence of new infections, i.e. a temporary or partial break in the chain of infection, but as the length of this stage is short, the probability of detection may also be lower **(Figures 14-15**).

Figure 14. Rates of *F. magna* infection stages in liver samples by hunting area in 2022/23 (n=125)

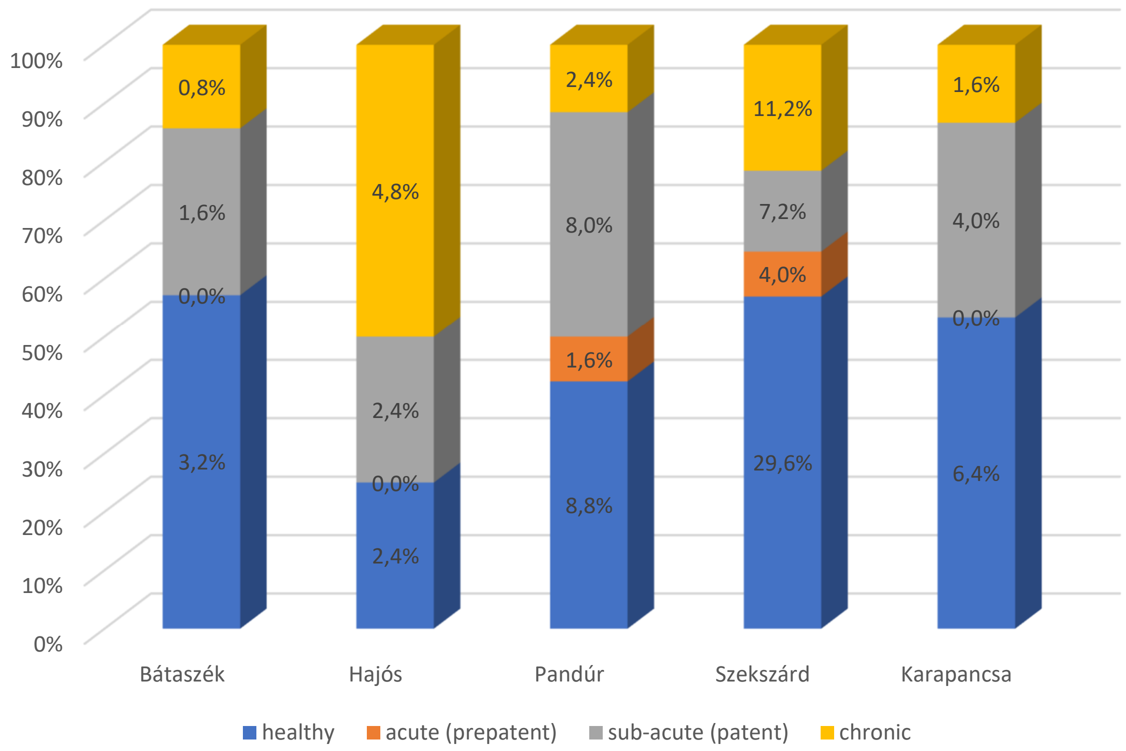
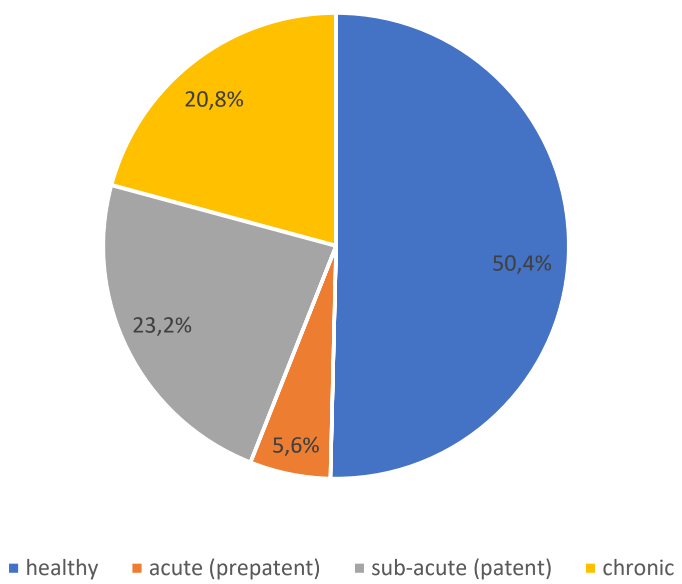


Figure 15. Overall rates of *F. magna* infection stages in 2022/23 (n=125)



In the second hunting year acute infections were found in all areas, indicating the occurrence of fresh infections and the proportion of sub-acute infections, indicating the current egg burden in the area and the expected intensity of re-infestation, was highest in Karapancsa, followed by the Pandúr hunting area. Due to the relatively small sample sizes, these proportion estimates should be treated cautiously in both study periods **(Figures 16-17).**

Figure 16. Rates of *F. magna* infection stages in liver samples by hunting area in 2023/24 (n=109)

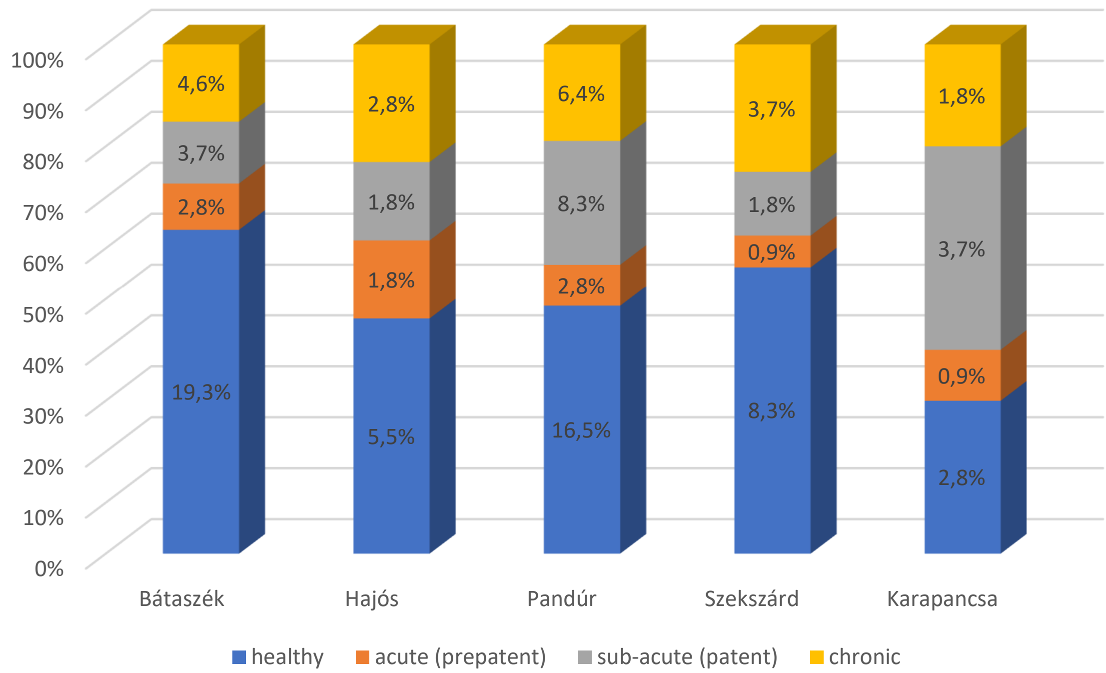
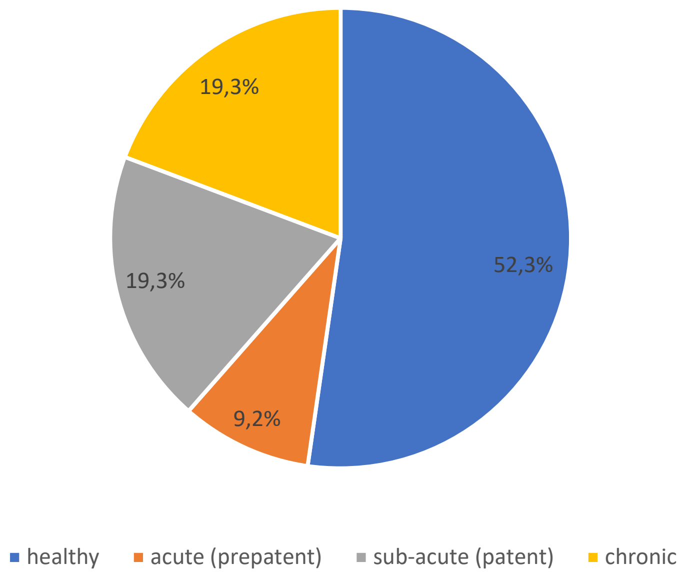


Figure 17. Overall rates of *F. magna* infection stages in 2023/24 (n=109)



4.1. Parasitology examination of liver samples containing flukes

Starting from early September 2022, flukes identified during the dissection of liver samples from red deer shot during that period, were subjected to further examination. Additionally, cavern-like structures or inclusions indicating the presence of fluke eggs. It was revealed through individual sample examinations that both flukes themselves and the brown cavern content consistently contained varying amounts of *Fascioloides* eggs. This clearly reveals that deer excrete eggs with their faces, indicating a chronic fluke infection, characterized by visible abnormalities in the liver that can be seen with the naked eye, whether alive flukes are present. The material surrounding the flukes or remaining after their death primarily consisted of amorphous material of the worms, i.e., digested blood, in which the eggs were suspended.

The flukes were separated and preserved from the samples, and the intact, undamaged specimens i.e., those that were not damaged during extraction, were prepared between two glass slides. The flukes obtained from different animals showed highly heterogeneous preservation, including completely intact specimens and liquified ones, even within the same sample. Since the samples could not be preserved before the examination to ensure the hatching ability of the eggs, it was impossible to accurately assess the condition of the worms at the time the deer were shot. Therefore, the examined flukes were categorized into only two groups: those obtained from deer shot during September-October and those obtained during November-December. More samples arrived for examination from the beginning of the study period than during the second half, resulting in fewer flukes in the latter group. Nevertheless, it was clearly demonstrated that the flukes in deer shot during September-October were smaller than those in deer shot during November-December, as illustrated by the prepared specimens (**Figure 18**).

Figure 18. Flukes found in early and late autumn

Note: The shape and colour of individual specimens primarily depend on the time elapsed between the shot and processing. On the left, flukes found in early autumn; on the right, flukes found in late autumn.

On the larger glass slide on the left side of **Figure 18**, you can see the flukes from deer shot during September-October, while on the smaller glass slide on the right side, you see those from deer shot during November-December. All the latter are egg-producing, fully mature fluke specimens, whereas among the former, the presence of eggs could be detected in about a quarter of the specimens through microscopic examination. Since there were no smaller specimens in the group consisting of larger flukes, and no notably larger specimens in the group consisting of smaller flukes, it can be inferred that the flukes uniformly grew over the months. This means that the infection occurred in the deer population more or less simultaneously, at least during the year 2022, within a relatively defined period, suggesting that the flukes entered the hosts' bodies within a confined timeframe. Considering that the developmental time for *Fascioloides magna* to become egg-producing specimens is roughly two and a half months, the infection of the examined deer can be estimated to have occurred around the latter half of July or the first half of August.

If this estimate is accurate, further conclusions can be drawn by considering the local weather conditions at that time and comparing it with when the forest management discontinued summer anthelmintic feeding. Since the year 2022 was relatively dry nationwide, it would be interesting to investigate how and where the deer could have become infected with this water-dependent parasite in the second half of the summer. If the locations of deer carcasses that perished in the winter of 2021 were recorded, the distribution of the carcass locations could provide some information about the areas where deer are most likely to get infected. These forest sections should be examined for snail habitats that did not dry out completely by the end of the last summer and the places where deer stayed after the period following anthelmintic feeding before the rutting season. Although larvae released from snails (metacercariae, and presumably eggs as well) can survive in the environment for a long time, the distribution of the flukes indicates that the occurrence of deer infection culminated within a specific period. If this is the case, it is not unreasonable to suggest that during that period, animals should receive an anthelmintic that is quickly eliminated from the body, primarily capable of destroying young flukes. In theory, this could prevent the flukes from producing eggs. Treatment applied in the spring or even at the end of winter can indeed eliminate the parasites themselves, but it cannot kill mature eggs accumulated in the liver. Therefore, the uniform development of *Fascioloides* flukes suggests that during further investigations, it would be worthwhile to determine the relatively defined and reasonably accurate period when young flukes develop in deer, for which shooting outside the hunting season would be suitable.

4.2. Parasitology examination of liver samples containing eggs

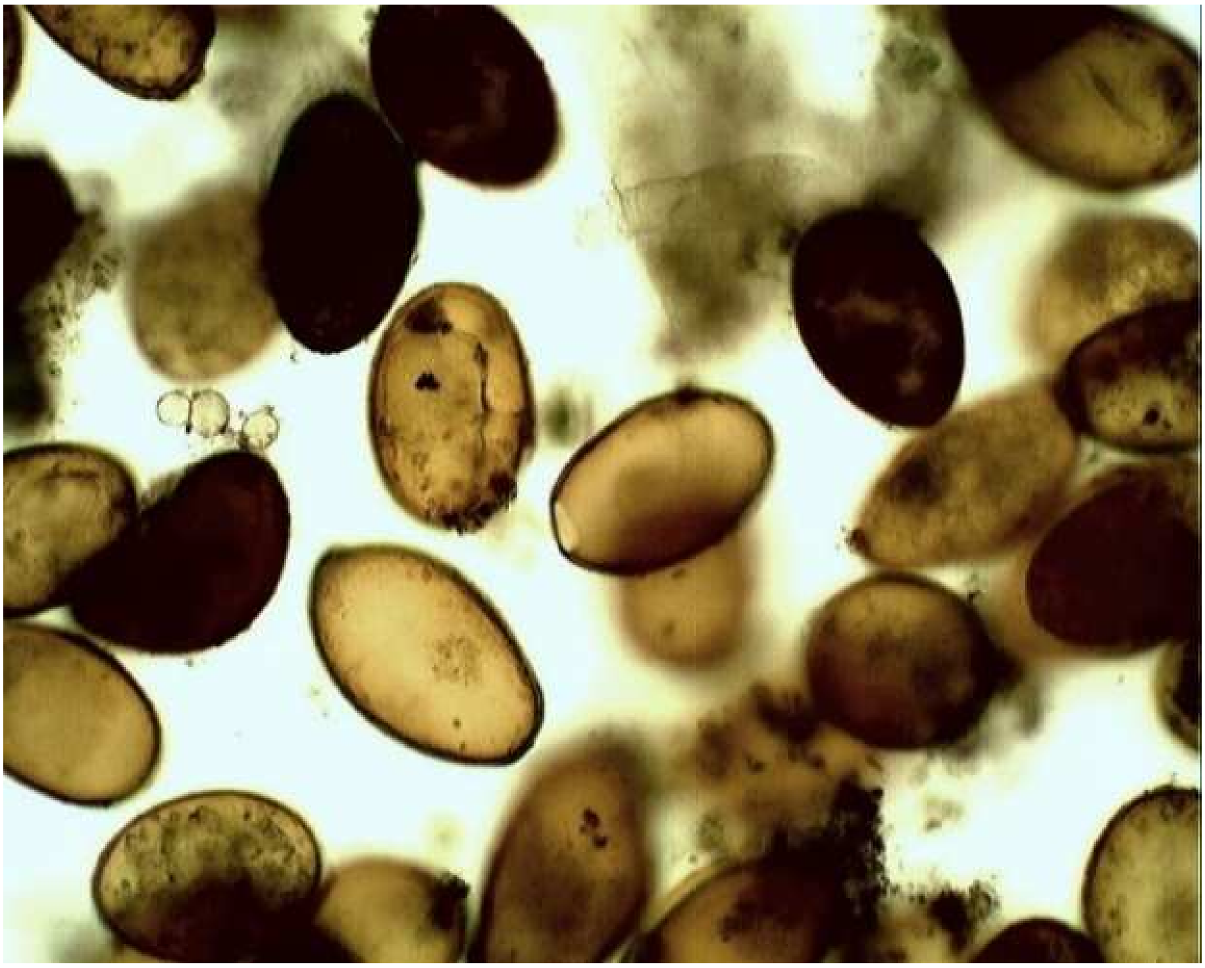
The other part of the study focused on assessing the viability of the eggs. The eggs were obtained from the amorphous decay products through repeated wet decantation, and in each sample, intact eggs containing viable zygotes were found. However, each sample primarily contained the mass of defective, dead eggs, which had already left the bodies of the flukes in distorted forms and had died in the caverns due to the lack of blood circulation, with only degenerated material present. Nevertheless, due to the vast quantity of eggs, there were always enough intact eggs in the material of the caverns, and these seemed capable of hatching.

Hatching began with the eggs extracted from the samples received in September, and a pool was formed for each month's samples. Hatching was consistently performed at a constant room temperature of 19°C, as even in a Hungarian forest swamp, ground temperatures do not exceed this level even in summer. At this temperature, the first miracidia appeared on the 28th day from the start of hatching, and fluke larvae continued to hatch evenly until the 35th-37th day. Each monthly sample required 28–30 days for the appearance of the first miracidia, but an unexpected development was that unhatched miracidia remained in the egg cases even 2-3 months after the onset of hatching (**Figure 19**). Although these miracidia appeared motionless at the beginning of microscopic examination, when exposed to the microscope lamp's radiation for several minutes, they started moving within the egg cases.

From October 2022, the eggs were obtained from samples underwater continuously. Occasionally examining these eggs, we still found eggs containing miracidia, although the majority of intact eggs only had their shells remaining. (Miracidia that hatch die within a day if they cannot reach a snail.) All this suggests that even eggs kept in a consistent microclimate do not hatch simultaneously, and the hatching process can be very prolonged.

To determine whether the eggs survive the winter in a viable state, approximately 2 million eggs were placed in 200 millilitres of water outdoors in early November. The eggs, mixed with tap water containing 1% glycerine, were buried in soil in a covered plastic container about 15-20 cm deep from the surface. This situation roughly simulates the condition in which eggs placed in decaying plant matter-containing mud may be exposed during the winter. Glycerine is intended to replace the cryopreservative effect of sapropel, and perforated top of the plastic container ensures semi-anaerobic conditions. We plan to examine the viability of the egg mass stored in this way in May, that is when we start attempting to hatch the eggs, as the approximate 10°C soil temperature is required for the activity of the intermediate host snails, and under natural conditions, encounters between snails and miracidia can be expected from this time onward.

Figure 19. Miracidium larvae



Note: In the centre-left of the image is a miracidium larva containing one or two pigment cells, and to its right is an empty egg capsule. At the top of the capsule, a round hole indicates where the miracidium hatched. The other eggs contain dead zygotes, degenerated cellular material, and the darkened shell of these eggs indicates the beginning of their decomposition.

Results from various international studies indicate that the common liver fluke (*Fasciola hepatica*) eggs remain viable outdoors during winter in countries with a temperate climate. Therefore, theoretically, it can be expected that *Fascioloides* eggs are similarly resistant. If it is confirmed that *F. magna* eggs can hatch even when overwintering outdoors in a zygote-containing state, then deer could still get infected in the summer even if they do not shed eggs after spring deworming. Therefore, a long-term examination of the viability of the eggs is necessary for shaping the strategy of treatment with medication.

Figure 20. The cross-section of a walnut-sized cavity shows only the eggs produced by them and the debris of the worms (no longer containing live flukes)

Note: The yellow-brown material in the centre of the cavity is dry, dense, and almost exclusively composed of fluke eggs. The hardened mass is isolated from the liver tissue by a layer of connective tissue, approximately half a centimetre thick.

A deer liver containing flukes was examined in a state where even the cavities in the parenchyma were not exposed. One of the densely packed cavities was cut in a way that preserved its internal structure, as the dead flukes' locations were already filled with condensed tissue debris. The centre of the cross-section of the cavity shown in **Figure 20** is filled with a mass of yellow-brown fluke eggs, framed by black coagulated deposits precipitated from digested blood. The cavity wall consists of a continuous layer of connective tissue that is so rigid that it retains the original shape of the cavity even when scraping out its contents (**Figure 21)**. Cavities of this type are always present in deer suffering from chronic fluke disease, and their significance lies in preserving the eggs within them even after the death of the flukes throughout the host animal's life.

Figure 21. Scraped contents of a cavity in the deer liver

Note: The scraped contents of the cavity in the deer liver demonstrate that the cavity's content strongly adheres to the connective tissue wall. The wall is composed of such dense connective tissue that it retains the protruding shape on the liver's surface even after the removal of the internal contents.

To demonstrate the viability of eggs in these cavities, scrapings were taken from the centre of the cavity, and the eggs obtained were subjected to hatching procedures. It was observed that even though in relatively small numbers, miracidia hatched from the eggs collected here. Since there are millions of eggs in a single cavity, the experienced hatching rate of 1-2% still indicates that a considerable number of fluke larvae can originate from a single cavity. The interior of these cavities was not reached by the effects of drugs; thus, the organs of animals suffering from chronic infection can remain infectious even if the drug eliminates all live flukes in them. Therefore, it should be prevented that eggs from fluke-infected deer livers enter the environment, whether treatment occurs or not.

Eggs containing fluke larvae exit the deer through the faeces into the external environment. However, eggs enclosed in the centres of the cavities can only be released from there if the liver is consumed raw by some animal or if the liver decomposes on the ground or possibly in the water. This can theoretically be prevented by disposing of the removed liver (and intestinal tract) during evisceration. Nevertheless, eggs entering the digestive tract and excreted with waste occur in infected deer throughout the year. If the flukes die, egg excretion can persist for at least several weeks. It is hoped that after effective treatment, the excretion of viable eggs will cease even if the eggs enclosed in the liver cavities remain alive. The investigation of this can theoretically be done in two ways. In one case, the viability of eggs can be examined in samples taken from the bile ducts of deer shot after treatment, and in the other case, the faces should be examined for the presence of eggs.

Despite the difficulty of collecting waste in a natural habitat, our suggestion is to collect waste samples on those feeding sites where drugs are placed for feeding to examine them for the presence of eggs at the beginning of treatment and well after the treatments to monitor the effects of the drugs. Since this would be too labour-intensive across the entire hunting area, we believed that as a first step in 2023, we had to collect at least 50, but possibly 100 waste samples from those feeding sites where the drug is deployed.

Starting from the beginning of September 2023, the content of cavities caused by flukes was further processed from the livers of deer shot during the hunting season. These cavities contained eggs and, in most cases, the flukes themselves. During the autopsy of the livers, 28 animals were found with such abnormalities, from which brown material could be collected from the caverna formed during chronic infection. In addition to these, there were cases of infections where only acute lesions without eggs or, conversely, chronic, old lesions from which eggs could no longer be extracted were found. As only the animals showing the latter types of lesions cannot be considered as spreaders from an epidemiological point of view, their livers without cavities were not subjected to parasitological examination.

Among the cavity contents examined, recognizable flukes or fragments of the parasite were found in only 15 cases. However, since all of these contained numerous *Fascioloides* eggs, it can be stated that all cavities were formed as a result of the action of these flukes. In total, 34 flukes were found in the samples, usually in the form of severed or decomposed specimens. Based on the fragments or whole flukes extracted, it could be determined that none of them were smaller than 4 centimeters, indicating that all were considered fully mature flukes. This means that most of them were already egg-producing flukes within the deer's liver at the beginning of the hunting season. No juvenile flukes were found, but if they were present in the dissected livers, they would not have been found in the cavities but rather in the damaged liver tissue.

The expected quantity of eggs, ranging in the hundreds of thousands, was found in each cavity. The livers and cavity samples were stored in refrigerators or deep freezers under anaerobic conditions, so larvae could no longer be cultured from the eggs. Nevertheless, microscopic examination of the eggs revealed which ones contained intact zygotes and which ones contained disintegrated content, indicating their non-viability. Furthermore, based on the colour of the eggshells, it could be determined whether there were immature eggs at the time of shooting, which originated from the disintegrated bodies of the flukes. These immature eggs suggested that the flukes were still alive and producing eggs at the time of shooting, as the mature eggs remain in the cavity content for a long time even after the death of the fluke, for example, due to the effect of a drug.

According to the examination of the eggs, all 28 cavity contents contained mature, intact eggs. Based on the examination of 100–100 eggs per sample, it was found that the proportion of intact eggs ranged from 55% to 85% compared to non-viable eggs, always exceeding 50%. Therefore, even with cautious estimation, intact eggs are present in the cavities in at least the tens of thousands, despite drug treatments.

The yellow colour of the eggshells develops in the body of the fluke over a period of approximately one to two weeks, so colourless, intact eggs can only be found in the flukes' bodies during the deer's life. The colourless eggs found during our examination were extracted from the fragmented or dissected bodies of the flukes during the autopsy. Such colourless eggs were found in 25 out of the 28 samples, indicating the presence of mostly sexually active, living flukes in the deer carrying the liver cavities. The experiences of the hunting year 2022-23 showed that older cavities generally contain more defective, damaged eggs than intact ones, although due to the massive quantity of eggs, this does not significantly affect the spread of intact eggs. The fact that there were more intact eggs than deformed, damaged eggs in the materials examined in 2023-24 suggests that the cavities examined were relatively newer compared to those in previous years. (Cavities persist in the host for years before hardening ("cheesifying") and turning into dead material.)

Since young flukes were not found in the cavity material, it can be assumed that the mass containing eggs was created by living, mature flukes, suggesting that these deer were infected in the summer of 2023 (or the infection occurred then), even before the start of the hunting season in September. The 28 egg-containing samples examined were obtained from the five hunting districts of Gemenc Inc., indicating the presence of deer shedding infective eggs in all of these areas (Bátaszék, Hajós, Pandúr, Szekszárd, and Karapancsa Hunting Districts).

From an epidemiological point of view, this means that regardless of the frequency and nature of pathological changes, the forest management area has a population containing animals shedding infective eggs, so the treatment must be continued. The containment of the epidemic could only be hoped for if the treated deer no longer shed viable eggs. This possibility would only be realized and demonstrable if only dead eggs were found in the cavities.

In theory, this state could be achieved by complete destruction of the flukes and demarcation ("closure") of the cavities. In this case, the animal would no longer shed eggs throughout its life, and the scatter of eggs in the cavities could be prevented by destroying the infected livers. Consistent with the results of examinations in the hunting year of 2022-23, it is highly likely that deer become infected with larvae emerging from snails at the end of summer. Therefore, the highest number of young flukes can be expected in the second half of summer, followed by the occurrence of mature flukes in the liver after the end of the hunting season. Thus, treatment with agents against mature flukes can begin immediately after the end of the hunting season, but preparations against young flukes should ideally be used during the summer (ideally in the second half).

Since there is a massive presence of *Fascioloides* eggs in the cavities, it is essential to consider what happens to them after the animal's death. It is crucial to remove the livers of shot animals from natural habitats and to destroy the livers in such way that viable eggs cannot scatter. The assumption that *Fascioloides* eggs overwinter in a living state is probably not true, as the eggs released into the environment at the beginning of the year failed to hatch. Therefore, deer become infected primarily through the release of eggs from spontaneous carcasses in the hunting area during the summer and the eggs excreted with the waste of live animals. Snail infections usually occur during the summer when they move and reproduce.

It is very difficult to find the snails themselves, and in the Gemenc area, only a few places are known where snails capable of carrying fluke larvae are definitely present. However, the quantity of fluke eggs in the waste can be estimated well, so if we had a picture of the distribution of infection based on faecal samples collected from the entire area, it might be possible to delineate the areas where snails are most likely to become infected.

4.3. Treatment protocols against *Fasciola magna* in Gemenc

Based on the prevalence data and the results of the parasitological examinations the University of Veterinary Medicine (UVMB) recommended a treatment based on the mechanism of benzimidazole-action, which involves either a single high dose or two consecutive daily doses. Slow-release, low-dose intake of substances is highly contraindicated due to inefficacy and the development of resistance. The administration of slow-acting, low-dose agents is strongly contraindicated due to ineffectiveness and the development of resistance. Therefore, the use of a more effective but more expensive protocol (triclabendazole and albendazole protocol) was proposed in Gemenc. On the basis of professional discussions based on the recommendations of the treatment protocol of UVMB, Gemenc Inc. decided to procure antiparasitic products containing triclabendazole (Tribex 10%) and albendazole (Albendanin 10%). These were mixed into salt licks and corn meals for distribution to deer.

For triclabendazole, Gemenc chose to mix it with ground corn pre-mix to ensure rapid and sufficient intake of the active ingredient. The recommended dosage for triclabendazole was a single application of 50 mg/kg body weight, assuming an average feed intake of 300 grams per animal, or 30 mg/kg body weight in two applications (with an interval of 2–4 days), assuming a similar intake. To counteract the bitter taste of triclabendazole, flavouring was essential, which included up to 5% salt and 5–8% vitamin C, based on the previous positive experiences of Gemenc Inc. After discussions, Gemenc Inc. decided on two applications of the active ingredient, and the deer almost completely consumed the corn-based feed containing triclabendazole. To treat 1,000 animals with two applications, a total of 650 kg of pre-mix was required, consisting of 400 kg of ground corn, 90 kg (litres) of Tribex 10% solution, 90 kg of silicate for stabilization, 30 kg of feed salt, and 40 kg of vitamin C for improved palatability.

For albendazole, Gemenc Inc. decided to mix it into salt lick powder. For 1,000 animals, assuming an average intake of 450 g of salt lick per animal, a total of 600 kg of salt lick mix was prepared. The treatment required 75 kg (litres) of Albendanin 10% solution, 30 kg of vitamin C for flavouring, and 45 kg of additives for texture, mixed with 450 kg of salt. The 600 kg of salt lick mix was distributed to the animals and was consumed in a short time, rendering the albendazole treatment successful in both the Gemenc and Karapancsa hunting areas.

5. Discussion

The introduction of *F. magna* was due to the translocation of bovine animals from North America into Europe [13] and was first described in the 20th century. The parasite distributed with the Danube River and until now it was described in the Czech Republic, Austria, Hungary, Germany, Slovak Republic, Croatia and Serbia [14, 15]. In the hunting area of Gemenc the overall prevalence of infection with *F. magna* for the whole red deer population varied between 40.4% and 50.0% in the two hunting years of the study, (2022-23 and 2023-24) which can be compared with similar Hungarian and international studies.

Another Hungarian study confirmed the existence of the Giant Liver Fluke in 2016 in the southern Transdanubian region of Hungary and a related study was done between 2018 and 2022 which revealed its presence with a prevalence of 68.4% in red deer [16]. In Croatia in the region of Spačva the prevalence of positive animals tested from 2007 to 2012 was 36.4%, including animals all ages with an 1:1 sex ratio [14]. This relates to our results as to its geographical location near the border of Serbia. In contrast to this, an Austrian study revealed that the prevalence of the Big American Liver Fluke was 0.03% in the snail host *Galba truncatula* during August 2004 and September 2005 in the Danube floodplain forests, which is related to the geographical distribution of *F. magna* [15]. In The Lower Silesian Wilderness, located in Poland and one of the biggest forests in Central-Europe, 99 red deer were shot during three hunting seasons. The parasitological findings showed the prevalence of 6.7% in 2014, 26.5% in 2016 and 28.6% in 2017. Similar to our study in Gemenc, all infected animals except one were adults [17]. In the Czech Republic a *F. magna* monitoring survey was done in 2009 and 2010 together with the "Czech Inspectors of Hunted Game" (CIHG) and altogether 345 deer samples were examined and only 8.4% were positive for *F. magna*. The CIHG who took place in the monitoring were evenly distributed in the country, but the majority of cases were located in the south-west of the Czech Republic, next to the German border [18].

The geographical occurrence of *F. magna* is highly dependent on the occurring climatic condition. For spreading an intermediate host, lymnaeid snails, are necessary, and they prefer standing waters and puddles [2, 19, 20]. The temperature plays a key role in the life cycle of the snails: higher temperature has a beneficial effect on development, reproduction and infectivity rates. According to this it is important to think of the global temperature rise, as the distribution and prevalence of fascioliasis may be affected [20]. Despite temperature, precipitation and humidity are other crucial factors for transmission and the severity of infection caused by the trematode. The parasite population may expand and proliferate, beyond their characteristic described enzootic area, with increasing temperatures and precipitation [13]. In the floodplain forests of the Danube River in Hungary muddy puddles are formed due to the heavy rainfalls and floodings and in these puddles the *Galba truncatula* as intermediate host for *F. magna* is populated densely on an annual basis. Wild living animals like red deer and wild boars wallowing in the puddles may carry the snails further away, creating a new habitat for the parasite [21].

Macroscopical changes in fluke infected livers from our study, starting from September 2022, showed cavern-like structures or inclusions, proving the presence of fluke eggs. Chronic fluke infections are revealed by the deer shedding fluke eggs with their faeces and characterized by visible, macroscopical abnormalities in the liver. More flukes were obtained from deer shot during autumn (September – November) than from deer shot during winter (November – December) and the flukes harvested in the beginning of the study period were smaller than the flukes at the end of it. It was shown that the flukes collected during the winter period were fully mature and egg producing in contrast to the other group, only in about a quarter of the fluke eggs were detected.

Similar pseudocysts were also found in livers of red deer in the study in the Transdanubian region of Hungary between 2018 and 2022, most of them contained two flukes, minimum one and maximum three fluke individuals were found inside. Additionally, egg production was observed inside these pseudocysts [16]. Compared to the findings in the Lower Silesian Wilderness, where 2 to 24 trematodes were isolated from red deer livers, the infection intensity is quite low [17]. In our study in Gemenc the number of flukes per infected deer ranged from 4.2 to 14 in the season from 2022/2023 with its highest prevalence in Karapancsa. Macroscopically Filip-Hutsch et al. found severe hepatic lesions and hepatomegaly in infected animals, regional lymph nodes were enlarged, and the diaphragmatic side of the liver was covered with fibrin and showed dark irregular lines. Similar to previous results, the cross-sections resembled thick-walled pseudocysts filled with flukes, partially dead ones, their eggs and brown liquid material. From each capsule one to six flukes could be identified, and smaller individuals were migrating through the liver parenchyma [17].

Infection stages can be characterized by pathological changes and can be divided into “acute infection”, “sub-acute infection” and “chronic infection”. In the acute infection the flukes are developing and migrating causing liver damage, but infected hosts do not shed eggs. In the sub-acute stage encapsulated fluke cysts and mature flukes can be seen, and active egg shedding is present. In the chronic stage the flukes itself died, but the cyst containing eggs may still be infective. Our study demonstrated that 50% of the liver samples were healthy, 23% in a sub-acute infection stage, 21% in a chronic infection stage and 6% acute infection stage in the examination during the first phase of the study. Similar overall rates of the *F. magna* infection stages were demonstrated in the examinations the year after, the fewest individuals were found in the acute infection stage. A main difference was that in the second hunting year of survey, in all examined areas acute infections were found, indicating the occurrence of fresh infections.

Halász et al. (2023) also examined *F. magna* infestation in roe deer. Similar histopathological lesions were observed in infected roe and red deer livers, including thick-walled pseudocysts, disrupted liver structure, and inflammatory changes. The study confirmed that *F. magna* can reach sexual maturity and produce fertile eggs in roe deer as aberrant host is possible, with eggs showing high survival potential [16]. In the Lower Silesian Wilderness 99 collected red deer livers showed cirrhosis, fibrosis and massive tissue damage [17].

In numerous studies coprological examinations of deer drooping were done. In the Lower Silesian Wilderness 170 samples were collected and *F. magna* eggs were found in more than 50% (53.4%) of the investigated red deer faces and these species were also found in fallow deer and roe deer material [17]. 30 years ago a case report was already published in Hungary about the Large American Liver Fluke in the Szigetköz area, between Dunaremete and Győr, which reveals 11 out of 15 faecal samples containing fluke eggs [22].

For a successful treatment of *F. magna*, a long-term examination of the viability of the eggs due to their resistance is necessary. It is mentioned that the most important task in the control of the large American liver fluke is the coprological monitoring in wet habitat where the fluke persists [23]. On the other hand, eggs from fluke-infected deer livers should be prevented entering the environment, whether treatment occurs or not. The most effective substance for treating *F. magna* is triclabendazole, and it should be administered in two consecutive days maximum. Treatment with medicated corn can be done once at the beginning and once at the end of winter [24]. In the Gemenc region, triclabendazole and albendazole were administered through corn meals and salt licks. It is proven that the treatment works in deer, difficulties are presented in the individual treatment and in the precise dosing if group treatment is administered [25]. As preventive therapy, individual treatment is the only reliable method to suppress the infection [23].

6. Summary

This thesis examines the prevalence, impact, and control of *Fascioloides magna* infections in red deer populations within the hunting areas of Gemenc Inc. in Hungary. The giant liver fluke represents a major parasitic threat to cervids, with implications for wildlife conservation and habitat sustainability. Over two hunting years (2022-23 and 2023-24), the study involved detailed parasitological and pathological examinations of liver samples to assess infection prevalence, characterize disease progression, and develop treatment and control strategies.

Results demonstrated a high prevalence of infection among adult red deer, 73.7% in the first examination period and 60.3% in the second period. Broken down by hunting areas, the prevalence rate was the highest in Hajós (82%) and the lowest in Bátaszék (60%) in 2022/2023 and in 2023/2024 it was Karapancsa (75%) and Hajós (55%), respectively. Notably, calves under six months were initially free of infection, but cases emerged among older calves in the second hunting year, suggesting increasing transmission within this age group. Across the study, there was no significant difference in infection rates between sexes: 74% infected stags and 73% infected does in 2022/2023 and 61% infected stags and 60% infected does in 2023/2024, with both does and stags showing similar infection patterns.

The study revealed variations in infection intensity and severity across hunting areas. Interestingly, infection dynamics suggested a defined transmission period, likely tied to seasonal environmental factors and the availability of intermediate snail hosts. This insight underscores the importance of timing anthelmintic treatments during critical transmission windows to prevent egg production and subsequent reinfestation.

The findings highlight the need for integrated management strategies, including targeted anthelmintic interventions (e.g., triclabendazole, albendazole) and habitat monitoring to disrupt the parasite’s life cycle. Recommendations include administering fast-acting treatments during periods of fluke maturation and investigating snail habitats in forest sections prone to infection.

7. References

1. Kassai T (1999) Veterinary helminthology. Butterworth-Heinemann, Oxford ; Boston

2. Malcicka M (2015) Life history and biology of Fascioloides magna (Trematoda) and its native and exotic hosts. Ecology and Evolution 5:1381–1397. https://doi.org/10.1002/ece3.1414

3. Deplazes P, Joachim A, Mathis A, Strube C, Taubert A, Samson-Himmelstjerna G von, Zahner H (2021) Parasitologie für die Tiermedizin, 4., überarbeitete Auflage. Georg Thieme Verlag, Stuttgart New York

4. Rochette F (n.d.) Dog parasites and their control. Beerse: Janssen animal health.

5. Stiles CW (1894) The Anatomy of the Large American Fluke (Fasciola Magna). J Comp Med Vet Arch 15:225–243

6. Juhásová L, Králová-Hromadová I, Bazsalovicsová E, Minárik G, Štefka J, Mikulíček P, Pálková L, Pybus M (2016) Population structure and dispersal routes of an invasive parasite, Fascioloides magna, in North America and Europe. Parasit Vectors 9:547. https://doi.org/10.1186/s13071-016-1811-z

7. Juhásová L, Králová-Hromadová I, Zeleňáková M, Blišťan P, Bazsalovicsová E (2017) Transmission risk assessment of invasive fluke Fascioloides magna using GIS-modelling and multicriteria analysis methods. Helminthologia 54:119–131. https://doi.org/10.1515/helm-2017-0016

8. Králová-Hromadová I, Bazsalovicsová E, Štefka J, Špakulová M, Vávrová S, Szemes T, Tkach V, Trudgett A, Pybus M (2011) Multiple origins of European populations of the giant liver fluke Fascioloides magna (Trematoda: Fasciolidae), a liver parasite of ruminants. International Journal for Parasitology 41:373-383. https://doi.org/10.1016/j.ijpara.2010.10.010

9. Vinarski MV (2016) Annotated type catalogue of lymnaeid snails (Mollusca, Gastropoda) in the collection of the Natural History Museum, Berlin. ZSE 92:131–152. https://doi.org/10.3897/zse.92.8107

10. Králová-Hromadová I, Juhásová L, Bazsalovicsová E (2016) Distribution of Fascioloides magna. In: The Giant Liver Fluke, Fascioloides magna: Past, Present and Future Research. Springer International Publishing, Cham, pp 17–40

11. Rehbein S, Visser M, Hamel D, Reindl H (2021) Occurrence of the giant liver fluke, Fascioloides magna, in sympatric wild ungulates in one area in the Upper Palatinate Forest (northeastern Bavaria, Germany). Parasitol Res 120:553–561. https://doi.org/10.1007/s00436-020-06996-7

12. Nagy E, Jócsák I, Csivincsik Á, Zsolnai A, Halász T, Nyúl A, Plucsinszki Z, Simon T, Szabó S, Turbók J, Nemes C, Sugár L, Nagy G (2018) Establishment of Fascioloides magna in a new region of Hungary: case report. Parasitol Res 117:3683–3687. https://doi.org/10.1007/s00436-018-6099-9

13. Hoberg EP (2010) Invasive processes, mosaics and the structure of helminth parasite faunas. Rev Sci Tech 29:255–272

14. Konjević D, Janicki Z, Calmels P, Jan DS, Marinculić A, Šimunović M, Pavlak M, Krapinec K, Poljak Z (2018) Evaluation of factors affecting the efficacy of treatment against Fascioloides magna in wild red deer population. Vet Ital 54:33–39. https://doi.org/10.12834/VetIt.970.5051.1

15. Hörweg C, Prosl H, Wille-Piazzai W, Joachim A, Sattmann H (2011) Prevalence of Fascioloides magna in Galba truncatula in the Danube backwater area east of Vienna, Austria. Wiener tierärztliche Monatsschrift 98:261–267

16. Halász T, Tari T, Nagy E, Nagy G, Csivincsik Á (2023) Hatchability of Fascioloides magna Eggs in Cervids. Pathogens 12:741. https://doi.org/10.3390/pathogens12050741

17. Filip-Hutsch K, Pyziel-Serafin AM, Hutsch T, Bulak K, Czopowicz M, Merta D, Kobielski J, Demiaszkiewicz AW (2022) The Occurrence of Fascioloides Magna (Bassi, 1875) in the Wild Cervid Population in the Lower Silesian Wilderness - Epidemiological and Pathological Aspects. J Vet Res 66:381–387. https://doi.org/10.2478/jvetres-2022-0042

18. Kasny M, Beran L, Siegelova V, Siegel T, Leontovyc R, Berankova K, Horak K (2012) Geographical distribution of the giant liver fluke (Fascioloides magna) in the Czech Republic and potential risk of its further spread. Veterinární medicína

19. Mas-Coma S, Valero MA, Bargues MD (2009) Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. Vet Parasitol 163:264–280. https://doi.org/10.1016/j.vetpar.2009.03.024

20. Dube A, Kalinda C, Manyangadze T, Mindu T, Chimbari MJ (2023) Effects of temperature on the life history traits of intermediate host snails of fascioliasis: A systematic review. PLoS Negl Trop Dis 17:e0011812. https://doi.org/10.1371/journal.pntd.0011812

21. Juhász A, Majoros G (2023) A proposed ectochory of Galba truncatula snails between wallow sites enhances transmission of Fascioloides magna at gemenc, in Hungary. Int J Parasitol Parasites Wildl 21:219–223. https://doi.org/10.1016/j.ijppaw.2023.06.004

22. Majoros G, Sztojkov V (1994) Appearance of the large American liver fluke Fascioloides magna (Bassi, 1875)(Trematoda: Fasciolata) in Hungary. Parasitologia Hungarica 27–38

23. Slavica A, Florijančić T, Janicki Z, Konjević D, Severin K, Marinculić A, Pintur K (2006) Treatment of fascioloidosis (Fascioloides magna, Bassi, 1875) in free ranging and captive red deer (Cervus elaphus L.) at eastern Croatia. Veterinarski arhiv 76:S9–S18

24. Lalor R, Cwiklinski K, Calvani NED, Dorey A, Hamon S, Corrales JL, Dalton JP, De Marco Verissimo C (2021) Pathogenicity and virulence of the liver flukes *Fasciola hepatica* and *Fasciola Gigantica* that cause the zoonosis Fasciolosis. Virulence 12:2839–2867. https://doi.org/10.1080/21505594.2021.1996520

25. Trailović SM, Marinković D, Kulišić Z (2016) Diagnosis and therapy of liver fluke (Fascioloides magna) infection in fallow deer (Dama dama) in Serbia. J Wildl Dis 52:319–326. https://doi.org/10.7589/2015-07-194

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