

Research article

Occurrence, ecology and management of *Fascioloides magna* in Bavaria, southern Germany

Andreas König  and Christian Ehrmantraut

Wildlife Biology and Management, Technical University of Munich, Freising, Germany

Correspondence: Andreas König (a.koenig@tum.de)

Wildlife Biology

2024: e01277

doi: [10.1002/wlb3.01277](https://doi.org/10.1002/wlb3.01277)

Subject Editor: Hideharu Tsukada

Editor-in-Chief: Ilse Storch

Accepted 07 May 2024



In 2015, red deer infected with American liver fluke were discovered in the Veldenstein Forest area for the first time. This was the first detection of the fluke in a wild deer population in Bavaria. The aim of the study was to determine the American liver fluke prevalence rates in red deer, roe deer and wild boar in the Veldenstein Forest, as well as factors influencing these rates. Since 2018, the livers of 83% of the red deer culled in the study area have been examined, as well as those of the occasional roe deer and wild boar. The livers are classified by adspsection and dissection into four levels of infection. The age of the animals was estimated based on dentition and tooth cementum annuli in the first molar (M1). The livers of 520 red deer, 226 roe deer and 75 wild boar were dissected. All wild boar livers tested negative. 3% of roe deer and 36% of red deer livers were positive. The red deer population was reduced to lower the prevalence, beginning in 2018. In the following years, it fell significantly. Medium and high infection levels were initially detected in 61% of adult red deer. Since 2018, the median number of flukes per infected liver has decreased significantly from 17.51 flukes/liver to 10.0 flukes/liver. On driven hunts, significantly more diseased deer were found than during hunting from raised hides. Furthermore, there are close correlations between the age of the red deer, infections, and the distance from the Pegnitz floodplains. The American liver fluke leads to the extinction of roe deer locally and to a massive infection and decimation of the red deer. Without a drastic reduction in the number of infected deer populations, there is a risk of large-scale infection of native deer species across Bavaria.

Keywords: disease management, large american liver fluke, prevalence rate, red deer, roe deer, wild boar

Introduction

Fascioloides magna or giant American liver fluke is the North American counterpart to *Fasciola hepatica*, the large or common liver fluke. *Fascioloides magna* was accidentally introduced to northern Italy in 1865 with wapitis brought to the 'La Mandria' enclosure near Turin ([Marincultic et al. 2002](#), [Kralova-Hromadova et al. 2011](#)). Around 1910, further accidental introductions brought the fluke with white-tailed deer and



www.wildlifebiology.org

© 2024 The Authors. Wildlife Biology published by John Wiley & Sons Ltd on behalf of Nordic Society Oikos

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

wapiti to Bohemia and Moravia in the Czech Republic, (Ullrich 1930, Kralova-Hromadova et al. 2011, Kasny et al. 2012) and to Slovakia (Erhardova-Kotrla and Kotrly 1968, Kralova-Hromadova et al. 2011). From there the parasite spread via contact with infected red deer and natural migration along the Danube (Bazsalovicsova et al. 2013) to Hungary (Majoros and Sztojkov 1994, Nagy et al. 2018), Austria (2000) (Hörweg et al. 2012, Sattmann and Hörweg 2006), and from 2000 also to Croatia (Marinculic et al. 2002, Konjevic et al. 2018) and Serbia (Malcicka 2015). Riverine landscapes such as those found along the Danube or other rivers such as the Pegnitz and Oder not only provide an excellent habitat for red deer (Raesfeld and Reulecke 1988, Bützler 2001), but also for *F. magna*, due to the presence of various snail species that serve as intermediate hosts (Vignoles et al. 2006, Sanabria et al. 2013). The Czech Republic can currently be seen as the source region for spreading this liver fluke to many neighbouring countries, with the exception of Italy. For some years now, it has been spreading towards Germany and Poland (Novobilsky et al. 2007, Kasny et al. 2012, Sattmann et al. 2014). As early as 2007, Novobilsky and colleagues pointed out that the liver fluke could spread to Germany in the area of the Bohemian Forest from the Šumava National Park (Novobilsky et al. 2007).

Czech veterinarians criticise the fact that too little attention is paid to the problem of *F. magna* in the Czech Republic (Kasny et al. 2012). It is, therefore, not surprising that there were almost simultaneous reports in 2015 of *F. magna* infections in fallow deer in enclosures near the Polish–Czech border in Poland (Demiaszkiewicz et al. 2015), and in red deer in enclosures along the Bavarian–Czech border near Flossenburg (Plötz 2015).

Of the hoofed game species in central Europe, red deer and fallow deer (Ullrich 1930) are regarded as definitive hosts (Csivincsik et al. 2023), wild boar, respectively feral hogs (Foreyt and Todd 1972) and sika deer as dead-end hosts, and roe deer as aberrant hosts (Juhász and Stothard 2023). In definitive hosts, the fluke goes through its normal life cycle, leading in central Europe to considerable mortality, as it causes severe weight loss and organ failure. It also impairs the reproductive capacity of the animals (Ursprung and Prosl 2011, Juhász and Stothard 2023, Csivincsik et al. 2023). Prevalence rates of between 29% and 100% are reported for red deer in various countries (Ursprung and Prosl 2011, Sattmann et al. 2014, Filip-Hutsch et al. 2022). Drug treatments have been shown to have some success with red deer in enclosures (Slavica et al. 2006). In freely roaming red deer on the floodplains of the Danube in Austria, the prevalence initially fell sharply, only to rise again to 80% from one year to the next (Ursprung and Prosl 2011). For drug treatment of the definitive host to be successful, each individual animal in a population must be treated. This is only possible for game in enclosures, and not for freely roaming populations (Slavica et al. 2006). As alternatives, measures such as reducing the red deer population (Ursprung and Prosl 2011) or keeping them away from areas suitable as habitats for the snails (Slavica et al. 2006) are described.

Aberrant hosts such as roe deer, sheep or goats die quickly, as the juvenile flukes migrate mainly through the liver parenchyma and destroy it in the process (Malcicka 2015). For roe deer, this appears to apply for areas newly infested with *F. magna*. Recent studies on roe deer in endemic areas show that pseudocyst development, egg production and egg shedding can occasionally occur in this host (Csivincsik et al. 2023). In addition, chronic infections and reinfections were detected in 34 roe deer examined in Croatia (Konjevic et al. 2021). This confirms initial reports from Poland on the formation of pseudocysts in roe deer (Demiaszkiewicz et al. 2018). From various European countries, prevalences of *F. magna* infection in roe deer ranging from 14% to 80% are reported (Csivincsik et al. 2023).

In dead-end hosts, the fluke completes its normal development, except that it cannot excrete eggs into the environment, as there is no connection between the pseudocysts and the bile ducts (Malcicka 2015).

The life cycle of *F. magna* is similar to that of *Fasciola hepatica*. The eggs are excreted into the environment with the faeces of the definitive hosts. The eggs mature in moist environments into larvae – the miracidia. These must find and penetrate a suitable intermediate host within one to two days. In Europe, this is usually the freshwater snail *Galba truncatula* (Hörweg et al. 2012) or, somewhat more rarely, the *Radix peregra* (Juhász and Stothard 2023). Prevalences of 0.003–0.2% in the snails are enough to trigger infection rates of between 20% and 100% in red deer (Sattmann et al. 2014).

Each miracidium in a snail develops into 300 to 600 (1000) cercariae (Mulvey et al. 1991), which are excreted from the snail into the water. They then attach themselves as cysts to a plant, losing their tail and becoming infectious metacercariae (Dunkel et al. 1996). When red deer ingest the infectious metacercariae, it takes 31 weeks for an adult, sexually mature fluke to develop from the metacercariae and enter the liver of the host animal (Foreyt and Todd 1976).

After migration of the juvenile stages through the liver parenchyma, the flukes form thinly walled pseudocysts (Plötz et al. 2015) in the final host. Here they reach sexual maturity. The pseudocysts are connected to the bile ducts, through which the flukes excrete their eggs (Plötz et al. 2015). Adult liver flukes (Fig. 1) can be between 8 and 10 cm long and up to 3 cm wide (Kasny et al. 2012, Deplazes et al. 2013).

As the freshwater snail *G. truncatula* occurs in habitats that are permanently or temporarily water-logged (Dunkel et al. 1996, Charlier et al. 2014, Juhász and Stothard 2023), it is widespread and occurs from low altitudes to the high mountains (Boch and Schneidawind 1988).

Since 2015, *F. magna* has also been observed in red deer in the area along the border between Upper Franconia and Upper Palatinate in the Veldensteiner Forest. Unusually, the nearest site of occurrence of the parasite in Bavaria is in and close to an enclosed wildlife reserve in the district of Neustadt an der Waldnaab, on the border with the Czech Republic (Plötz et al. 2015, Rehbein et al. 2021), and thus about

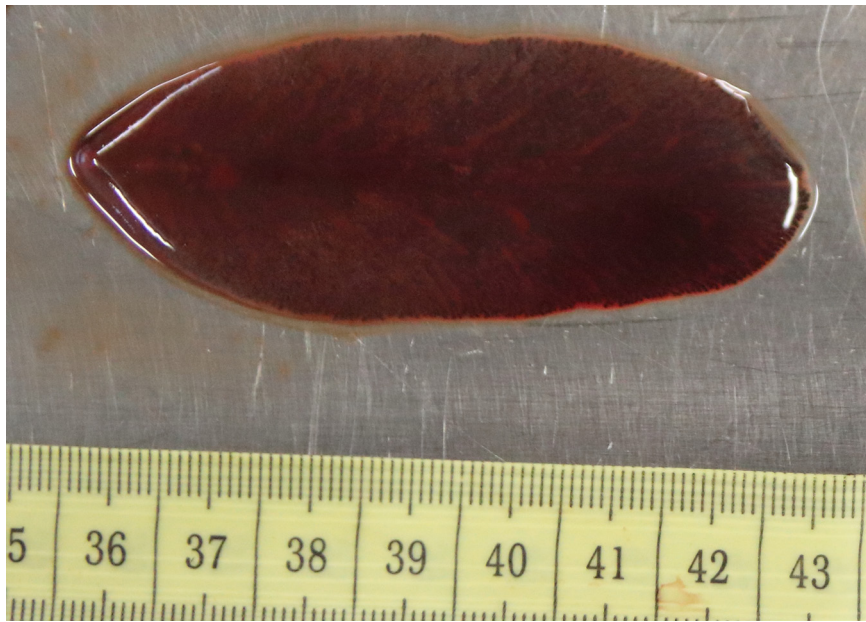


Figure 1. Mature fluke.

70 km away from this site as the crow flies. Genetic analyses have nevertheless shown that the *F. magna* in the Veldenstein Forest originates from the Czech population (Baumgartner 2020). This liver fluke has not been detected between the two sites of occurrence in the Veldenstein Forest and in the Czech Republic, and it has only migrated from the Veldenstein Forest into the neighbouring military training area to the east and thus towards the Czech border in the last few years.

To document the prevalence of *F. magna* and determine the ecological aspects of its occurrence, nearly all red deer shot in the region since 2018 were tested for liver flukes. For the roe deer and wild boar, only random samples were tested for liver flukes. To prevent the further spread of *F. magna*, the culling target for red deer has significantly increased since 2018 to reduce the population. The aim of this reduction in the population was to lower the prevalence in line with recommendations from the literature (Ursprung and Prosl 2011). Accordingly, the following research hypotheses can be formulated for the study:

1. As well as red deer, roe deer and wild boar are also infected by the liver fluke. There is no difference in prevalence among red deer, roe deer and wild boar (H1)
2. The prevalence can be reduced by reducing the red deer population (H2)
3. As the freshwater snail *G. truncatulla* can be found throughout the study area, there are no local differences in prevalence (H3)
4. Due to multiple infections, both the rate and intensity of infection increase with age (H4)
5. In the detection of infected red deer, there is no correlation between the hunting method and prevalence in the animals shot using each method (H5)

Material and methods

Study area

The study area is located in growth district 6.1, in the northern Franconian Jura (Nördliche Frankenalb) and northern Upper Palatinate Jura (Nördlicher Oberpfälzer Jura), at an altitude of 350–650 m a.s.l., with precipitation of up to 810 mm per year and an average annual temperature of 7.3°C (Arbeitskreis-Standortskartierung 1985). The poorer sites are predominantly stocked with pine and mixed pine stands, while beech and spruce stands can be found where the nutrient supply is better. The forested area comprises some 5000 ha to 6000 ha and is interrupted in the east by the meadows of the Pegnitz valley. These wet meadows are adjoined by a resting zone for red deer, so red deer are often found in the meadows.

Sampling

The samples are collected in the course of normal hunting activity. Each hunter records data on every animal shot for differentiated analysis:

- The culling of the animal
 - Site of culling
 - Date
 - Time of day
 - m above sea level
 - Method of hunting
- Animal killed
 - Game species
 - Sex
 - Age group

- Condition of the animal
 - External appearance – healthy/diseased
 - Weight after killing
 - Weight field-dressed, with head and legs
 - Hind leg length

The age of the animals was determined by tooth section, on the basis of the *cementum annuli* of the M1 (Mitchel 1967), and the lower jaw lengths and hind leg lengths of the specimens were used as indicators of skeletal development (Langvatn 1977).

Examination of the material

All of the livers were subjected to adspersion, palpation and dissection. The livers were cut into 2 cm-wide strips (Fig. 3) for dissection (Konjevic et al. 2018). The samples were divided into four categories of findings:

No signs of infection

Healthy liver with no change in colour or structure (Fig. 2).

Low-level

A few migratory tracks with black pigment dots or their traces are more or less recognisable on the surface of the liver (Fig. 4). The liver surface and liver tissue are largely intact. In the liver parenchyma, minor damage (migratory tracks, encapsulated flukes, tissue residues, haemorrhages) is present in the tissue (Fig. 5).

Medium level

Some migration channels with black pigment dots and their traces are clearly recognisable on the surface of the liver. The liver surface and liver tissue show clear changes in colour and structure. Blister-like structures may be visible on the surface and in the liver tissue. There is clear damage (migratory tracks, encapsulated flukes, tissue residues, haemorrhages) in the tissue (Fig. 6).

Figure 7 shows a liver cut into two centimeter-thick slices, with a pseudocyst (A) and several migratory tracks (B). The liver lies in a pool consisting of tissue residues, digestive material of the flukes and haemorrhaged blood.

High level

In this category, numerous migratory tracks with black pigment dots and their traces are recognisable on the surface of the liver. The surface of the liver shows severe changes in colour and structure, and numerous blister-like structures are visible on the surface and in the liver tissue. The liver parenchyma shows massive damage (migratory tracks, encapsulated flukes, tissue residues, haemorrhages) in the tissue (Fig. 8).

Number of flukes

The individual flukes are collected during dissection. The sieves in the drain of the dissection table are then searched for flukes.

Intervention to reduce the number of red deer

The first results of the study in 2018 made clear that the infection of the red deer population with *F. magna* was more advanced than had been assumed. In order to reduce the prevalence and prevent further spread of the fluke, attempts were made to decimate the red deer population. In 2018, the hunting bag increased to 275 red deer, corresponding to 143% of the long-term average. In the hunting year 22/23, only 63 animals were killed. The red deer were shot during the regular hunting seasons and within the context of the regular shooting plan. Additional licences from the hunting authorities were therefore not required.

Statistics

SPSS 27 was used for statistical calculations. Mean value comparisons were tested for significance using the t-test, and Levene's test was used to check the equality of variances (F). Differences in mean values among several groups were



Figure 2. No signs of infection (no. 70 green).



Figure 3. Liver cut into 2 cm slices without signs of infection (no. 70 green).

tested using analysis of variance (ANOVA). Factors influencing the intensity of infection were determined using linear regression and binary regression. Two-sided 95% confidence intervals were calculated in accordance with Cannon and Roe (1990).

Results

Over the entire study period, 520 samples were collected from red deer, 226 from roe deer, and 75 from wild boar.

The red deer samples analysed represent 83% of the red deer killed by hunters during the study period. On the basis of this, a prevalence of 35 % (CI 33.5%–41.0%) was calculated for red deer over the entire study period. On average across all years, the prevalence in roe deer is 3% (CI 1.0%–6.5%). There was no difference in prevalences between 2018 and 2020 (2%). Only in 2021 did the prevalence rise to 11%, before falling to 0% the following year (Fig. 9). The prevalence of *F. magna* in red and roe deer differed significantly ($p < 0.001$).

No wild boar samples showed signs of infection.

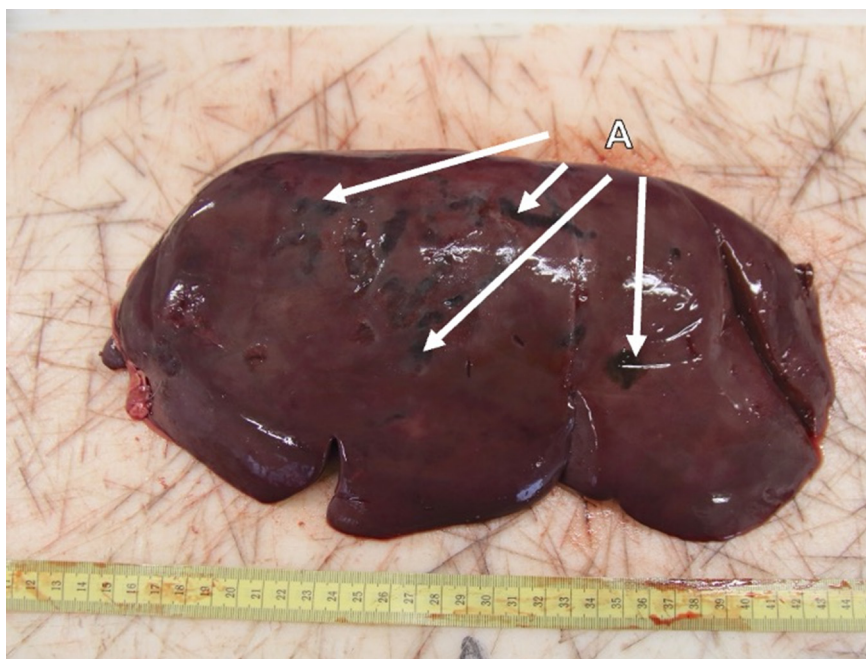


Figure 4. Low level of infection (A = migratory tracks).

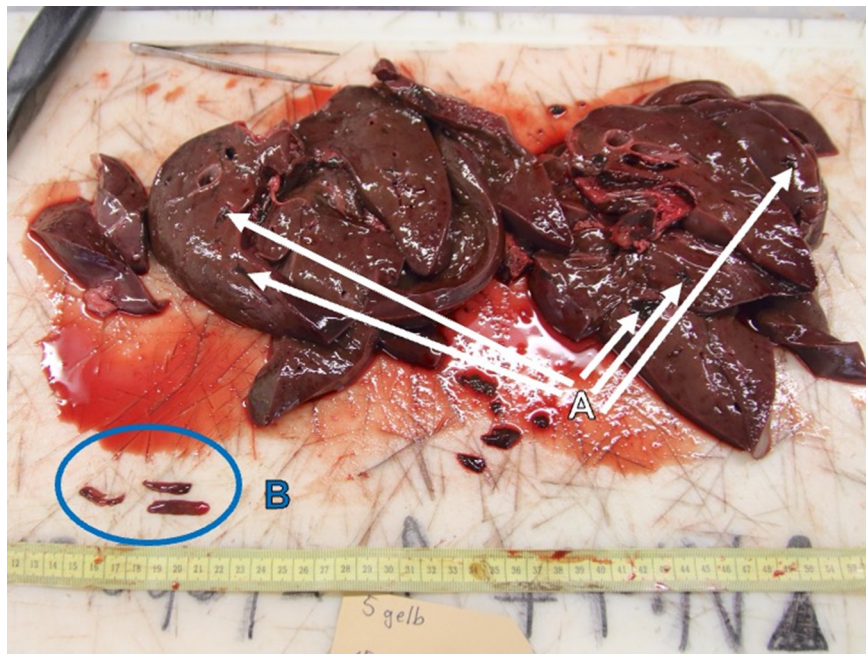


Figure 5. Liver cut into 2 cm slices with low level of infection (A=migratory tracks, B=three liver flukes *Fascioloides magna*).

In red deer, the prevalence decreased from 41% originally (CI 35–48%) in 2018 to 27% (CI 20–35%) (2019), 34% (24–46%) (2020) and 30% (CI 15.0–48.5%) (2021), rising again to 41% (27.5–55.8%) in the 2022 hunting year. The confidence intervals of the prevalences in red deer per year of the study overlap. The mean values of the annual prevalences do not differ significantly ($F=2.124$, $p=0.077$).

If, in the case of red deer, we consider all red deer excluding the calves, 51% ($n=343$; CI 34.2–38.4%) are infected

(**Fig. 10**). If we reduce the data set to only the adult animals (not including the yearlings), the prevalence rises to 62% ($n=230$; CI 69.2–79.7%) over the study period.

The differences in prevalence between hinds and stags (61% and 60%, respectively) are not significant ($p=5.93$).

If we look at the highest prevalence in adult red deer differentiated according to 'before the major intervention in the population' (2018) and 'after the major intervention' (2019 to 2022), 72% (CI 63.5–81.0%) on average were found to

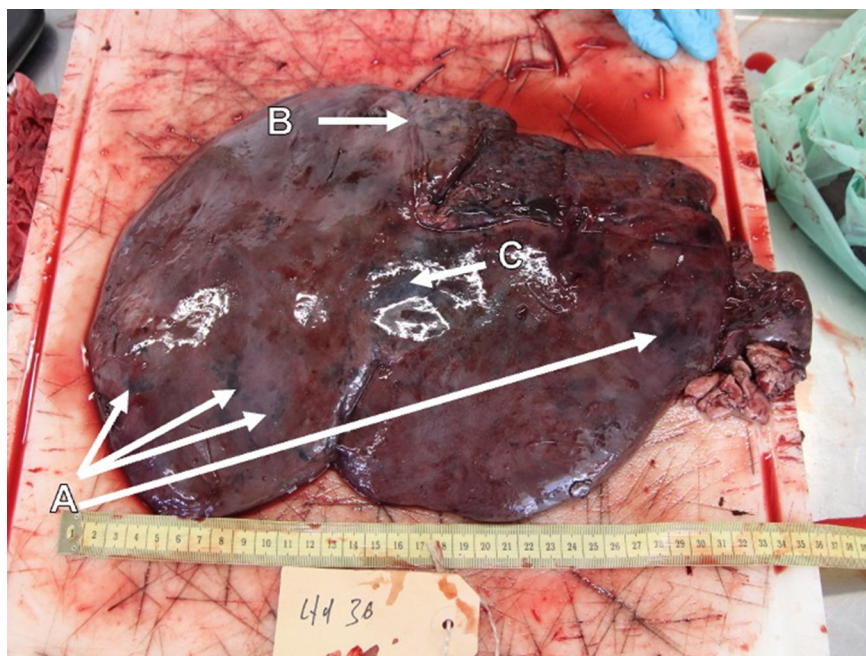


Figure 6. Medium level of infection (A=migratory tracks, B + C=Blister-like structure).

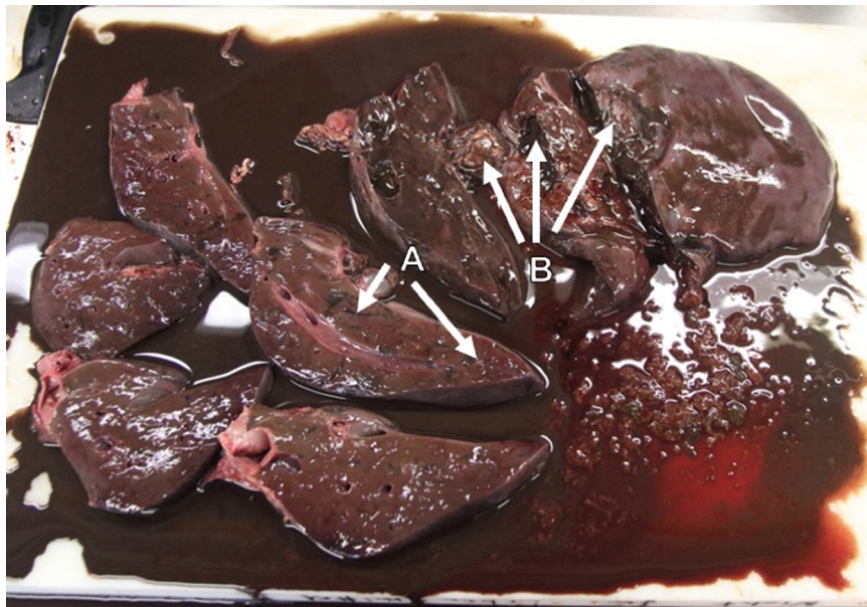


Figure 7. Medium-level of infection. (A=pseudocyst, B=migratory tracks).

be positive for liver fluke 'before' (2018) and 51% (CI 46.2–62.0%) 'after' (2019–2022). The decrease in prevalence in adult red deer ($F=30.340$ $p < 0.001$; $t=3.312$, $df=227.705$, $p=0.001$) is significant. If we include the one-year-old animals as well as the adult animals, the prevalence drops from 61% (CI 54–69%) 'before' to 44% (CI 36–51%) 'after' the intervention. This reduction in prevalence is also significant ($F=2.572$ $p=0.110$; $t=3.184$, $df=245$, $p=0.002$).

Seen over the entire study period, 20% of the red deer had a low-level of infection, 11% a medium-level, and only 6%

a high-level of infection (Fig. 11). No high levels of infection were detected in calves. A positive significant correlation exists between the number of flukes per infected liver and the degree of infection ($p < 0.001$). In high-level infections, an average of 47.18 (SD 24.895) flukes was found per liver. In this group, the highest number of flukes found in one liver was 109. A low-level of infection shows minor superficial changes and also an average of 3.91 (SD 3.734) flukes per liver, whereas in medium-level infections an average of 16.13 (SD 9.202) flukes are present in the liver. The mean number

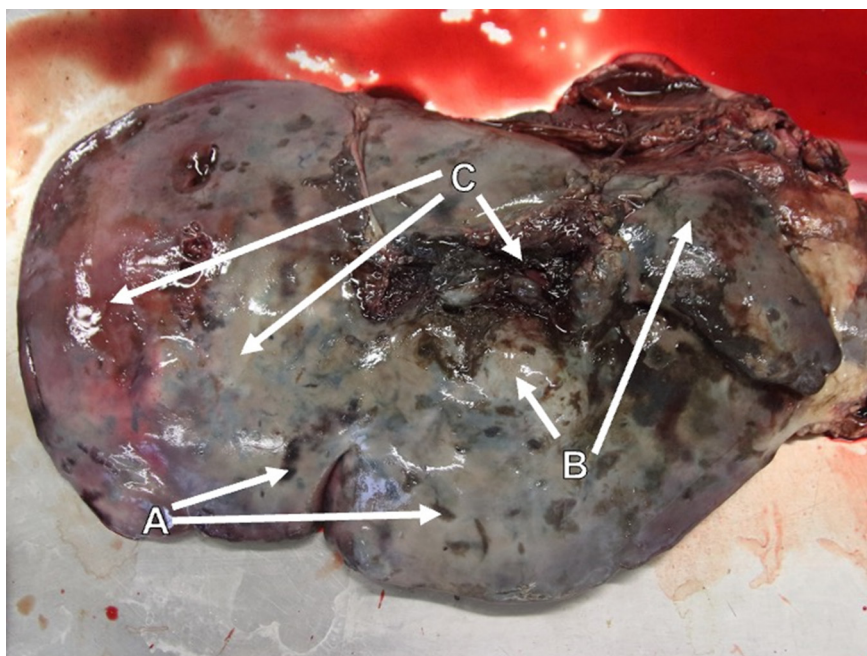


Figure 8. High level of infection (A=migratory tracks; B=blister-like structure; C=changes in colour and structure).

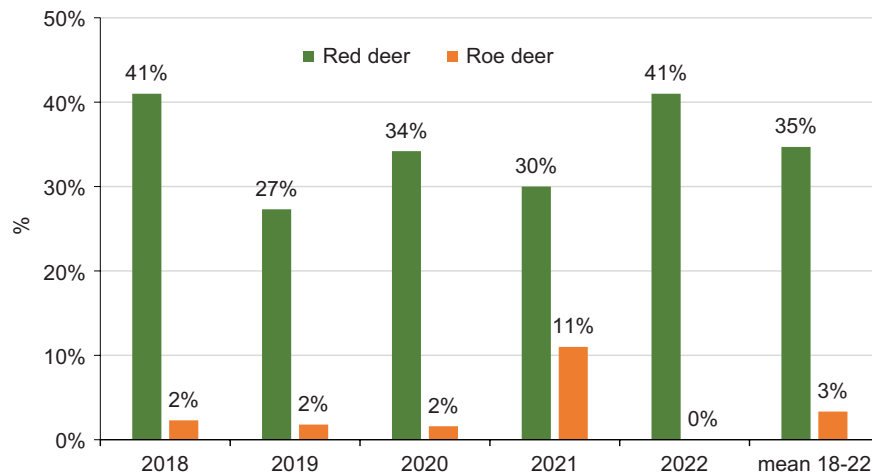


Figure 9. Change in prevalences in red deer and roe deer between 2018 and 2022.

of flukes per level of infection differed significantly between the low and medium-level categories ($p < 0.001$), medium and high-level categories ($p < 0.001$), and low and high-level categories ($p < 0.001$).

On average across all levels of infection, a red deer is infected with 14.78 flukes. The number of flukes per infected liver decreased from an average of 17.51 flukes per liver in 2018 to 10.0 flukes per liver in 2022 ($F = 5.516$, $p = 0.021$; $t = 2.475$, $df = 64.498$, $p = 0.016$).

The number of liver flukes per animal not only increases with the level of infection, but is also dependent on age. The number of flukes thus correlates significantly with the age of the red deer ($r = 0.183$ and $p = 0.015$).

In roe deer, a mean infection intensity of 2.25 (SD 2.500) flukes per infected liver was found. The highest number of flukes found in a roe deer liver was six.

As the distance to the River Pegnitz increases, the prevalence of liver fluke in red deer decreases. In the wooded areas close to the Pegnitz, the prevalence is around 54%, whereas

in the areas far from the Pegnitz it is only 11% ($F = 130.136$, $p < 0.001$; $t = -6.504$; $df = 148.548$; $p < 0.001$).

As there is a significant correlation between the variables 'distance from the Pegnitz' and 'age' and infection, a binary logistic regression (backwards, Wald) was calculated between infection (yes/no), age, distance from the Pegnitz and the sex of the deer (Table 1).

The age of the red deer and the distance from the River Pegnitz have significant influence on the prevalence rate.

A linear regression (backwards) was calculated to test whether the age of the red deer and the distance from the River Pegnitz also have an influence on the intensity of infection in the red deer (Table 2).

The best model for explaining the intensity of infection with the liver fluke contains only the 'age' variable.

The liver fluke has a direct influence on the condition of the animals. The mean weight of the adult red deer that tested negative is 68.8 kg, whereas the mean weight for those that tested positive for *F. magna* is significantly

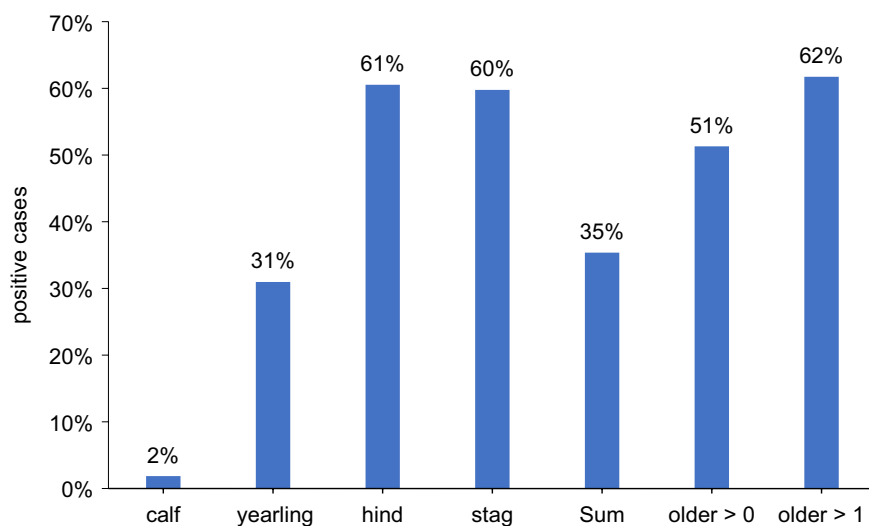


Figure 10. Prevalences in red deer differentiated according to age group.

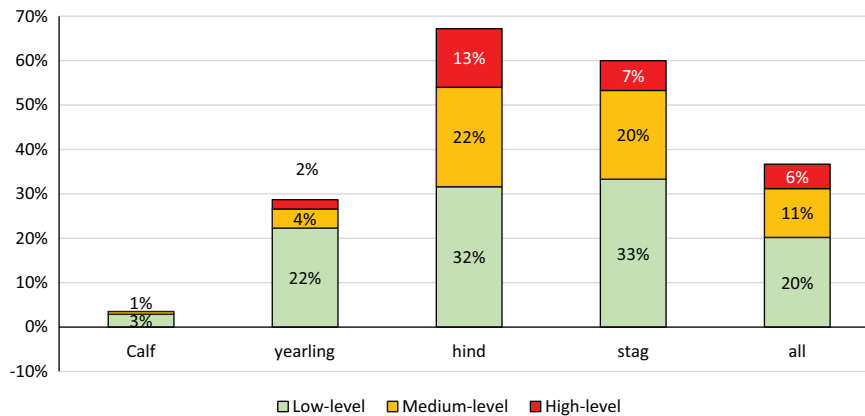


Figure 11. Infection levels differentiated according to age group.

lower, at 61.4 kg ($F = 3.067$, $p = 0.082$; $t = 2.469$, $df = 132$, $p = 0.015$).

Differentiated according to the method of hunting, 44% of the infected animals were culled on driven hunts and 28% on hide hunts ($t = 3.484$; $df = 347.863$; $p < 0.001$).

Discussion

Since 2018, 83% of the red deer shot by hunters in the Veldenstein Forest, as well as some individual roe deer and wild boar, have been tested for *F. magna*. While the occurrence of infection with *F. magna* in red deer in the Veldenstein Forest is well represented due to the high volume of data recorded, the sampling is unsystematic and random in the case of roe deer and wild boar, except when it comes to driven hunts.

Compared with other European studies, the prevalences in red deer found here appear to be within the normal range, at an average of 35%. Prevalence rates in red deer of between 20% and 100% are reported from Croatia, the Czech Republic, Austria and Hungary (Marinculic et al. 2002, Janicki et al. 2005, Ursprung and Prosl 2011, Kasny et al. 2012, Sattmann et al. 2014, Nagy et al. 2018). In comparison with these studies, the prevalences found here are at the lower end of the scale. In other studies, however, information on the age of the animals from which samples are taken is rarely provided, and systematic recording, as done here for red deer, was only carried out in the studies in Austria. If all samples from red deer one year old and older are taken into account, the prevalence rises to 51%. If only red deer older than one year are taken into account, the prevalence is 62%. Excluding calves,

Konjevic et al. (2018) found a prevalence of 46% along the Danube in Croatia (Konjevic et al. 2018). In comparison with this, our results are slightly higher.

A very low prevalence of 3% on average was found in roe deer. These results are relatively low in comparison with other European countries. Prevalences of 36.7% are reported in roe deer in Hungary (Halasz et al. 2023), for example, and 13.6% in Poland (Houszka et al. 2016). However, it should also be noted that only 22 roe deer were examined here.

Fascioloides magna could not be detected in the 75 wild boar samples. The infections reported in Europe were mainly in deer species. In the USA there were however also reports of infections in feral hogs. In a study of 29 feral hogs in Texas, for example, a prevalence of 51.7% was found (Foreyt and Todd 1972). The fact that no infection was found in wild boar in our study may have to do with the area of infection. Red deer and roe deer become infected in the Pegnitz floodplain area (König 2023) by ingesting infected grasses. Wild boars can barely utilize grasses for energy, so they look for soil insects to eat when on the Pegnitz floodplains.

Research hypothesis H1 can therefore be confirmed for red deer and roe deer in the Veldenstein Forest, but not for wild boar.

It was possible to initially reduce the prevalence of 41% in the red deer population of the Veldenstein Forest at the beginning of the project to 27%, with major intervention to reduce the red deer population. It rose again to 34% in the following year before falling back to 30%. It then rose again to 41% in 2022. At the same time, there was a decrease in the number of samples analysed. The study was launched in 2018 with 223 samples. In 2019, the number of samples fell to 134. It fluctuated between 75 and 30 samples per year from 2020 to 2022. These small sample sizes result in very wide

Table 1. Results of the binary logistic regression.

	Variables included					
	Regression coefficient	SD	Wald	df	p-value	Exp(B)
Age	0.341	0.042	64.527	1	< 0.001	1.407
Distance from Pegnitz	-0.259	0.69	13.890	1	< 0.001	0.727
Constant term	-0.967	0.205	22.138	1	< 0.001	0.380

Table 2 Results of the regression model.

	Variables included				
	Regression coefficient B	Standard error	Beta	t	p-value
Age	1.080	2.443		2.550	0.012
Constant term	9.083	0.424	0.195	3.718	< 0.001

confidence intervals, meaning no change can be detected. As it was too early for the increased culling of animals to have an effect on the prevalence in 2018, the prevalence in 2018 can be compared with the prevalences after the start of the culling to reduce the population, from 2019 to 2022, in order to estimate the influence of the measure on the incidence of infection. A comparison of the adult animals shot in 2018 with those shot from 2019 to 2022 thus reveals a significant decrease in the infection rate of adult red deer, from 72% to 51%. If the sample size considered is increased to include the one-year-old animals, the prevalence also decreases significantly from 63% in 2018 to 44% in the following years. In both cases, the confidence intervals do not overlap. This also supports a decrease in prevalence. This means that working hypothesis H2 can also be confirmed. However, studies of a white-tailed deer population showed only a weak correlation between population density and the incidence of infection (Vanderwaal et al. 2015). This is consistent with our results, in that we were able to document a reduction in prevalence, but the infection cycle was not interrupted.

However, the assumption that the population has declined sharply is based on the hunting bag, and not on an estimate of the population. The decline in the population is supported by the fact that in comparison with previous years, the hunting bag increased by 47% in the hunting year of 2018. It was noticeable during this period that only a few adult animals had fawns with them. This could either be due to a decline in fertility caused by the fluke (Csivincsik et al. 2023, Juhasz and Stothard 2023), or to the presence of the wolf (Smith et al. 2004). Furthermore, animals with a high level of infection are also easier prey for wolves.

The reduction of the red deer population in the Veldenstein Forest could not wholly prevent infection with *F. magna* in the red deer population there, but the reduced prevalence and reduction in the number of flukes per infected red deer have slowed down its spread. The mobile hunting methods used are likely to have had a major influence here. The combination of the higher hunting bag, the hunting method, and the preying of wolves is probably responsible for the successful reduction in prevalence and the lower number of flukes per infected liver. At the same time, the results show that the reduction process needs to be maintained over a long period. This is because a fluke can survive for over 5 years in the liver, and when it dies, the infected game will continue to excrete its eggs for several years (Deplazes et al. 2013).

The reduction, therefore, complies with the legal stipulations of Articles 1 and 33 of the Bavarian hunting law, which aim to maintain species-rich and healthy game populations through hunting (Schuck et al. 2019).

As repeatedly demanded by some people, the treatment of wild animals with medication is not legally permitted in

Germany. Moreover, although medication has had an effect on populations in enclosures (Slavica et al. 2006, Trailovic et al. 2016), it has only led to a reduction in prevalence in wild red deer populations, and in some cases it cannot prevent the prevalence rising again (Ursprung and Prosl 2011). Comparisons between red deer in enclosures and freely roaming red deer have shown that success can only be achieved with medication where each individual animal is treated (Janicki et al. 2005, Slavica et al. 2006). This is not possible with freely roaming red deer.

In contrast to the findings in Croatian studies (Konjevic et al. 2018), a correlation was found in the Veldensteiner Forest between the local prevalence and the distance from the floodplains of the River Pegnitz. Prevalence decreased with increasing distance from the wet meadows on the floodplains, thus disproving research hypothesis H3. This is consistent with findings from North America, where an increased risk of infection in wetlands was also found for wapiti *Cervus elaphus canadensis* and white-tailed deer *Odocoileus virginianus* (Mulvey et al. 1991, Pybus et al. 2015, Vanderwaal et al. 2015). The results are nevertheless surprising, given that the freshwater snail *Galba truncatula* was detected throughout the entire area of the Veldenstein Forest in the context of a study on detecting water-bound species. In this eDNA study, by contrast, we could only detect *F. magna* in the Pegnitz floodplains, which would support the rejection of research hypothesis H3 (König 2023). It is apparently not only the presence of intermediate hosts that is necessary for the infection process, but also their presence in sufficient numbers in correspondingly large wet meadows and/or water areas where red deer can go to drink. Keeping the red deer away from potentially infected areas or wetlands where the snail occurs is thus often suggested as an effective measure (Slavica et al. 2006, Trailovic et al. 2016).

Our data show a close relationship between the age of the red deer and the prevalence rate and intensity of infection, supported by the correlation and regression calculations. Prevalence thus increased with age, beginning with a prevalence of 2% in calves, rising to 31% in one-year-olds, and to 61% in adult red deer. At the same time, there were no 'highly' infected animals found among the calves. This is consistent with findings from North America, where the prevalence and intensity of infection in wapiti was also found to be dependent on the age of the game (Pybus et al. 2015). The results are also supported by studies from the Czech Republic, where an increase in prevalence rates with age was also found. The increase in prevalence with age can be explained on the one hand by multiple infection of the animals. In some infected livers, for example, both subadult and adult flukes were found. Subdominant red deer are rarely found in the floodplains, but as Pegnitz floodplains are the area of infection,

the probability of them becoming infected during their occasional visits here also increases over the years.

Between 2 and 144 flukes per liver were found in infected red deer in the studies in the Czech Republic (Erhardova-Kotrla and Kotrly 1968). This finding is also in line with our results, in which between 2 and 109 flukes were detected per infected liver. Only age was found to be significant as an explanatory variable for the intensity of an infection in the regression model. To summarise, research hypothesis H4 can thus also be affirmed for red deer.

According to the literature, as few as 6 flukes can lead to death in roe deer (Erhardova-Kotrla and Kotrly 1968). In our roe deer, just one liver fluke caused in a high severity of symptoms, resulting in severe destruction of the liver. As is the case with other aberrant hosts such as goats or sheep, it is assumed that roe deer will die as a result of infection with the parasite (Malcicka 2015, Csivincsik et al. 2023, Juhasz and Stothard 2023).

Also, with an average of 7.59 flukes per liver (Halasz et al. 2023), significantly more flukes were found in roe deer in Hungary than in our study. In our study, the highest number of flukes found in one roe deer liver was 6. The average was 2.25 flukes per liver. In three roe deer in Hungary, the formation of pseudocysts was detected (Halasz et al. 2023). This is consistent with earlier observations from Croatia (Konjevic et al. 2021). In the Veldenstein Forest, however, it does not yet appear to be the case that pseudocysts are developing in roe deer. Foresters on the floodplains along the River Pegnitz thus report that although there were considerable numbers of roe deer living alongside red deer some 15 years ago, there have been no more roe deer there since the occurrence of *F. magna*. None of the roe deer found to be positive was shot in the immediate vicinity of the floodplains. One reason for the overall low prevalence of *F. magna* in roe deer in our area may be due to the high population density of red deer in the Pegnitz floodplains and the resulting displacement effect on roe deer (König 2018). Due to the low rate of infection in roe deer, hypothesis 4 cannot be tested for roe deer.

Also associated with the infection and its intensity is a reduction in body weight. In adult red deer, the field-dressed body weight is reduced from an average of 68.8 kg to 61.4 kg. The massive damage to the liver can explain this result. Several times when samples were taken from red deer with a high-level of infection, blackish brown liquid escaped from the abdominal cavities of the animals in the course of field dressing, and the diaphragm was found to be black as a result of the destruction of the liver. Because of this potentially catastrophic damage caused to the liver by infection with *F. magna*, the literature states that infection can be fatal not only in roe deer but also in red deer (Foreyt and Todd 1972, Ursprung and Prosl 2011, Kasny et al. 2012, Sattmann et al. 2014, Csivincsik et al. 2023, Juhasz and Stothard 2023). It is also reported that reproduction declines in infected areas (Juhasz and Stothard 2023) something which can also be attributed to the poorer condition of the animals (König and Ehrmantraut 2023).

When it comes to hunting, drive hunting has proven its worth as a means of reducing prevalence. A disproportionately high number of infected animals are shot with this hunting method. This is due to the diseased animals' somewhat slower flight behavior, making them easier to harvest on driven hunts. The targeted culling of diseased animals from hunting hides fails because the symptoms of the disease are not identifiable from the animal's appearance.

Acknowledgements – The Pegnitz forestry operation of the Bavarian State Forests supported the project by providing the samples and funding.

Funding – The project was financially supported by Bayerische Staatsforsten AöR, Forstbetrieb Pegnitz, between 2019 and 2021. Sampling has been carried out by Bayerische Staatsforsten AöR, Forstbetrieb Pegnitz, since 2018.

Author contributions

Andreas König: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Methodology (equal); Project administration (lead); Resources (equal); Software (lead); Supervision (lead); Validation (equal); Visualization (lead); Writing – original draft (lead). **Christian Ehrmantraut:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (equal); Resources (equal); Validation (equal); Visualization (supporting).

Transparent peer review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/wlb3.01277>.

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.5x69p8dcj> (König and Ehrmantraut 2024).

References

- Arbeitskreis-Standortskartierung (Working group on site mapping) (ed.) 1985. Forest growth areas and growth districts in the Federal Republic of Germany (Forstliche Wuchsgebiete und Wuchsbezirke in der Bundesrepublik Deutschland). – Landwirtschaftsverlag GmbH, in German.
- Baumgartner, T. 2020. Investigation of the genetic diversity of *Fascioloides magna* in the red deer in Bavaria (*Cervus elaphus*). (Untersuchung zur genetischen Diversität von *Fascioloides magna* im bayrischen Rothirsch (*Cervus elaphus*)). – Bachelor thesis, Universität Hohenheim.
- Bazsalovicsova, E., Králová-Hromádová, I., Radvánszky, J. and Beck, R. 2013. The origin of the giant liver fluke, *Fascioloides magna* (Trematoda: Fasciolidae) from Croatia determined by high-resolution melting screening of mitochondrial cox1 haplotypes. – Parasitol. Res. 112: 2661–2666.

- Boch, J. and Schneidawind, H. 1988. Diseases of game. – Paul Parey. in German.
- Bützler, W. 2001. Red deer: Biology, behavior, environment, management. – BLV Verlag, in German.
- Cannon, R. M. and Roe, R. T. 1990. Infection control on life stock (Krankheitsüberwachung in Tierbeständen). (Lorenz, R. J., trans.). – AID, in German.
- Charlier, J., Soenen, K., De Roeck, E., Hantson, W., Ducheyne, E., Van Coillie, F., De Wulf, R., Hendrickx, G. and Vercruysse, J. 2014. Longitudinal study on the temporal and micro-spatial distribution of *Galba truncatula* in four farms in Belgium as a base for small-scale risk mapping of *Fasciola hepatica*. – Parasites Vectors, 7: 528.
- Csivincsik, Á., Halász, T. and Nagy, G. 2023. The large American liver fluke (*Fascioloides magna*): a survivor's journey through a constantly changing world. – Parasitologia 3: 300–326.
- Demiaszkiewicz, A. W., Kuligowska, I., Pyziel, A. M., Lachowicz, J. and Kowalczyk, R. 2015. Extension of occurrence area of the American fluke *Fascioloides* in south-western Poland. – Ann. Parasitol. 61: 93–96
- Demiaszkiewicz, A. W., Kowalczyk, R., Filip, K. J. and Pyziel, A. M. 2018. *Fascioloides magna*: a parasite of roe deer in Bory Zielonogórskie. – Med. Weter. 74: 6037–2018.
- Deplazes, P., Eckert, J., Samson, V., Himmelstjerna, G. and Zahner, H. 2013. Textbook of Parasitology for Veterinary Medicine (Lehrbuch der Parasitologie für die Tiermedizin, 3 ed. – Enke Verlag, in German.
- Dunkel, A. M., Rognlie, M. C., Johnson, G. R. and Knapp, S. E. 1996. Distribution of potential intermediate hosts for *Fasciola hepatica* and *Fascioloides magna* in Montana, USA. – Vet. Parasitol. 62: 63–70
- Erhardova-Kotrla, B. and Kotrly, A. 1968. Accidental introduction of a parasite of the genus *Fascioloides* with the import of live game from other continents. – Europ. J. Wildl. Res. 14: 170–176.
- Filip-Hutsch, K., Pyziel-Serafin, A. M., Hutsch, T., Bulak, K., Czapowicz, M., Merta, D., Kobielski, J. and Demiaszkiewicz, A. W. 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. doi:10.2478/jvetres-2022-0042.
- Foreyt, W. J. and Todd, A. C. 1972. The occurrence of *Fascioloides magna* and *Fasciola hepatica* together in the livers of naturally infected cattle in south Texas, and the Incidence of the fluke in cattle, white-tailed deer, and feral hogs. – J. Parasitol. 58: 1010–1011.
- Halász, T., Tari, T., Nagy, E., Nagy, G. and Csivincsik, Á. 2023. Hatchability of *Fascioloides magna* eggs in cervids. – Pathogens 12: 741. https://doi.org/10.3390/pathogens12050741.
- Hörweg, C., Prosl, H., Wille-Piazzai, W., Joachium, A. and Sattmann, H. 2012. Prevalence of *Fascioloides magna* in *Galba truncatula* in the Danube backwater area east of Vienna, Austria. – Wiener Tierärz. Monatschr. 98: 261–267
- Houszka, M., Piekarska, J., Podkowik, M., Gorczykowski, M. and Bania, J. 2016. Morphology and molecular study of *Fascioloides magna* – a growing threat to cervids (*Cervidae*) in Poland. – J. Vet. Res. 60: 435–439.
- Janicki, Z., Konjević, D. and Severin, K. 2005. Monitoring and treatment of *Fascioloides magna* in semi-farm red deer husbandry in Croatia. – Vet. Res. Commun. 29 Suppl 2: 83–88.
- Juhász, A. and Stothard, J. R. 2023. The giant liver fluke in Europe: a review of *Fascioloides magna* within cervids and livestock with considerations on an expanding snail-fluke transmission risk. – Adv. Parasitol. 119: 223–257.
- Kasny, M., Beran, L., Siegelova, V., Siegel, T., Leontovyc, R., Berankova, K., Pankrac, J., Kostakova, M. and Horak, P. 2012. Geographical distribution of the giant liver fluke (*Fascioloides magna*) in the Czech Republic and potential risk of its further spread. – Vet. Med. 57: 101–109.
- König, A. 2018. 12 years of hoofed game monitoring in the Ebersberg Game park (12 Jahre Schalenwild-Monitoring im Ebersberger Wildpark). – In: König, A., Arnold, J., Suchant, R. and Sandrini, M. (eds), Practical applications in wildlife ecology research - from monitoring to management (Wildtierökologische Forschung für die Praxis - vom Monitoring bis zum Management), Vol. 3. – Kesselverlag, in German.
- König, A. 2023. Monitoring aquatic species using eDNA (Monitoring wassergebundener Arten mittels eDNA). Retrieved from Freising, in German.
- König, A. and Ehrmantraut, C. 2023. Pregnancy of chamois does. – BaySE, pp. 22–23, in German.
- König, A. and Ehrmantraut, C. 2024. Data from: Occurrence, ecology, and management of *Fascioloides magna* in Bavaria, Southern Germany - Data. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.5x69p8dcj>.
- Konjevic, D., Janicki, Z., Calmels, P., Jan, D. S., Marinculić, A., Šimunović, M., Pavlak, M., Krapinec, K. and Poljak, Z. 2018. Evaluation of factors affecting the efficacy of treatment against *Fascioloides magna* in wild red deer population. – Vet. Ital. 54: 33–39.
- Konjevic, D., Bujanic, M., Beck, A., Beck, R., Martinkovic, F. and Janicki, Z. 2021. First record of chronic *Fascioloides magna* infection in roe deer (*Capreolus capreolus*). – Int. J. Parasitol. Parasites Wildl. 15: 173–176. doi:10.1016/j.ijppaw.2021.05.006.
- Konjevic, D., Bujančić, M., Beck, A., Beck, R., Martinković, F. and Janicki, Z. 2021. First record of chronic *Fascioloides magna* infection in roe deer (*Capreolus capreolus*). – Int. J. Parasitol. Parasites Wildl. 15: 173–176.
- Kralova-Hromadova, I., Bazsalovicsova, E., Stefka, J., Spakulova, M., Vávrová, S., Szemes, T., Tkach, V., Trudgett, A. and Pybus, M. 2011. Multiple origins of European populations of the giant liver fluke *Fascioloides magna* (Trematoda: Fasciolidae), a liver parasite of ruminants. – Int. J. Parasitol. 41: 373–383.
- Langvatn, R. 1977. Criteria of physical condition, growth and development in Cervidae, - suitable for routine studies. – Nordic Council for Wildlife Research.
- Majoros, G. and Sztojkov, V. 1994. Appearance of the large American liver fluke *Fascioloides magna* (Bassi, 1875) (Trematoda: Fasciolata) in Hungary. – Parasitol. Hungarica 27: 27–38.
- Malcicka, M. 2015. Life history and biology of *Fascioloides magna* (Trematoda) and its native and exotic hosts. – Ecol. Evol. 5: 1381–1397.
- Marincultic, A., Dzakula, N., Janicki, Z., Hardy, Z., Lucinger, S. and Zivcnjak, T. 2002. Appearance of American liver fluke (*Fascioloides magna*, Bassi, 1875) in Coratia – a case report. – Vet. Arh. 72: 319–325
- Mitchel, B. 1967. Growth layers in dental cement for determining the age of red deer (*Cervus elaphus* L.). – J. Anim. Ecol. 36: 279–293.
- Mulvey, M., Aho, J. M., Lydeard, C., Leberg, P. L. and Smith, M. H. 1991. Comparative population genetic structure of a parasite (*Fascioloides magna*) and its definitive host. – Evolution 45: 1628–1640.
- Nagy, E., Jocsak, I., Csivincsik, A., Zsolnai, A., Halasz, T., Nyul, A., Plucinszki, Z., Simon, T., Szabo, S., Turbok, J., Nemes, C., Sugar, L. and Nagy, G. 2018. Establishment of *Fascioloides*

- magna* in a new region of Hungary: case report. – Parasitol. Res. 117: 3683–3687. doi:10.1007/s00436-018-6099-9.
- Novobilsky, A., Horácková, E., Hirtová, L., Modrý, D. and Koudela, B. 2007. The giant liver fluke *Fascioloides magna* (Bassi 1875) in cervids in the Czech Republic and potential of its spreading to Germany. – Parasitol. Res. 100: 549–553.
- Plötz, C. A. 2015. Surveys to detect the large American liver fluke *Fascioloides magna* in game in enclosures in the north-eastern Upper Palatinate, and to detect infection with other endoparasites (Erhebungen zum Nachweis des Großen Amerikanischen Leberegel *Fascioloides magna* bei Gehegewild in der nordöstlichen Oberpfalz sowie zum Befall mit weiteren Endoparasiten). – PhD thesis. Ludwig-Maximilians-Universität München, in German.
- Plötz, C., Rehbein, S., Bamler, H., Reindl, H., Pfister, K. and Scheuerle, M. C. 2015. *Fascioloides magna* - epizootiology in a deer farm in Germany. – Berl. Munch. Tierarztl. Wochenschr. 128: <http://vetline.de/open-access/158/3216/>.
- Pybus, M. J., Butterworth, E. W. and Woods, J. G. 2015. An expanding population of the giant liver fluke (*Fascioloides magna*) in elk (*Cervus canadensis*) and other ungulates in Canada. – J. Wildl. Dis. 51: 431–445.
- Raesfeld, F. V. and Reulecke, K. 1988. The red deer. – Paul Parey Verlag, in German.
- Rehbein, S., Visser, M., Hamel, D. and 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.
- Sanabria, R., Mouzet, R., Pankrác, J., Djuikwo Teukeng, F. F., Courtioux, B., Novobilský, A., Höglund, J., Kašný, M., Vignoles, P., Dreyfuss, G., Rondelaud, D. and Romero, J. 2013. *Lymnaea neotropica* and *Lymnaea viatrix*, potential intermediate hosts for *Fascioloides magna*. – J. Helminthol. 87: 494–500.
- Sattmann, H. and Hörweg, C. 2006. Investigations to measure the infestation rate of the liver fluke *Galba truncatula* with the American giant liver fluke *Fascioloides magna* in the Danube Floodplain National Park. – Retrieved from Vienna, in German.
- Sattmann, H., Hörweg, C., Gaub, L., Feix, A. S., Haider, M., Walochnik, J., Rabitsch, W. and Prosl, H. 2014. Wherefrom and whereabouts of an alien: the American liver fluke *Fascioloides magna* in Austria: an overview. – Wien. Klin. Wochenschr. 126 Suppl 1: S23–S31.
- Schuck, M., Ellenberger, J., Frank, B., Koch, M., Von Massow, F., Munte, B., Schneider, R., Seibel, M., Tausch, A., Welp, G. and Wetzel, H. 2019. Federal Hunting Act with the inclusion of state law. Commentary. (Bundesjagdgesetz unter Einbeziehung des Landesrechts). Kommentar, 3. ed. – Verlag Franz Vahlen, in German.
- Slavica, A., Florijancic, T., Janicki, Z., Konjevic, D., Severin, K., Marinculic, A. and Pintur, K. 2006. Treatment of fascioloidosis (*Fascioloides magna*, Bassi, 1875) in free ranging and captive red deer (*Cervus elaphus* L.) at eastern Croatia. – Vet. Arh. 76: 9–18.
- Smith, D. W., Drummer, T. D., Murphy, K. M., Guernsey, D. S. and Evans, S. B. 2004. Winter prey selection and estimation of wolf kill rates in Yellowstone National Park, 1995–2000. – J. Wildl. Manage. 68: 153–166.
- Trailovic, S. M., Marinković, D. and 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.
- Ullrich, K. 1930. Occurrence of little known parasites in mammals and birds in Bohemia and Moravia. (Vorkommen wenig bekannter Parasiten bei Säugetieren und Vögeln in Böhmen und Mähren). – Prager Arch. Tiermedizin Vgl. Pathol. A 10: 19–43, in German.
- Ursprung, J. and Prosl, H. 2011. Occurrence and eradication of the American giant liver fluke (*Fascioloides magna*) in the Austrian Danube floodplains east of Vienna from 2000–2010. – Wiener Tierärztliche Monatschr. 98: 275–284.
- Vanderwaal, K. L., Windels, S. K., Olson, B. T., Vannatta, J. T. and Moen, R. 2015. Landscape influence on spatial patterns of meningeal worm and liver fluke infection in white-tailed deer. – Parasitology 142: 706–718.
- Vignoles, P., Novobilský, A., Rondelaud, D., Bellet, V., Treuil, P., Koudela, B. and Dreyfuss, G. 2006. Cercarial production of *Fascioloides magna* in the snail *Galba truncatula* (Gastropoda: Lymnaeidae). – Parasitol. Res. 98: 462–467.