**Fascioloides magna** and other liver parasites in cloven-hoofed game from northeastern Bavaria, Germany: occurrence and pathological findings with special emphasis on red deer (*Cervus elaphus*)

Marie Franziska Sommer1 · Juliana Drdlicek1 · Matthias Müller1 · Andrea Thelemann1 · Frank Thomas Just1

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**Abstract**

The first detection of *Fascioloides* (*F.*) *magna* in northeastern Bavaria in 2011 was presumably correlated to natural migration movements of free ranging wild ruminants originating from the neighboring Czech Republic, where high infection rates have been reported frequently. To gain more data on the continuing spreading and current occurrence of the giant liver fluke in surrounding regions, 700 livers of cloven-hoofed game originating from eleven different northeastern Bavarian counties were investigated for the presence of *F. magna* and accompanying liver parasites in the hunting season 2019–2020. Macroscopically altered liver tissue was further investigated by pathohistological and parasitological examination. *F. magna* was detected in 5.9% (38/640) of livers from red deer (0.7% < 1 year, 9.8% > 1 year; *p* < 0.05) whereas none of the investigated livers of wild boar, roe, and fallow deer was infected (*n*=60). Mild pathological alterations of the liver tissue were documented in 15, moderate in 14, and major in 9 of all *F. magna*-positive cases. Histologically, the fluke-specific pigment haematin, large trematode eggs, and periportal fibrosis were detected in the liver tissue of infected animals. In 9% of all investigated livers, parasitic stages of other parasites, i.e., *Dicrocoelium dendriticum* (6.0%), *Fasciola hepatica* (0.3%), *Taenia* spp. (1.7%), and *Echinococcus multilocularis* (1.0%), were found. According to the results of this study, *F. magna* is not restricted to formerly known affected regions of Upper Franconia, but is also present in the military training ground Grafenwöhr, Upper Palatinate, and the Bavarian Forest National Park, Lower Bavaria, with estimated prevalences of 3.6 and 16.4%, respectively, and thus seems to spread in suitable habitats in northeastern Bavaria.

**Keywords** Liver fluke · Red deer · Hepatic damage · Sedimentation · Trematode eggs

**Introduction**

Since the introduction of the giant liver fluke, *Fascioloides* (*F.*) *magna* (Bassi 1875) from North America, the trematode has established itself in numerous European countries starting off in Italy (Bassi 1875), the Czech Republic, and Slovakia (Erhardová-Kotrlá 1971; Kasny et al. 2012; Rajsky et al. 2002) spreading out to Austria (Sattmann et al. 2014), Croatia (Janicki et al. 2005), Hungary (Majoros et al. 1994; Nagy et al. 2018), and just recently to Germany (Plötz et al. 2015, Rehbein et al. 2021). Using different water snails of the family *Lymnaeidae* as intermediate hosts, the highly pathogenic fluke infects not only wild ruminants but also a variety of other mammals showing a variable pathogenicity depending on the affected definitive host species (Erhardová-Kotrlá 1971). Severe pathologic liver changes with fatal course of disease have been described for infected roe deer (*Capreolus capreolus*), sheep (*Ovis aries*), and goats (*Capra hircus*) whereas red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) might carry high numbers of the trematode correlated with a considerable destruction of liver tissue without showing any clinical signs (Ursprung et al. 2006). After the arrival of *F. magna* in northeastern Bavaria in 2011 (Rehbein et al. 2021), high infection rates have recently been reported in the red deer population in the Veldensteiner forest, Upper Franconia (König et al. 2019), leading to the assumption that the trematode might also be present in wild ungulates living in bordering counties. The increasing prevalence for *F. magna* in red deer living in certain regions in Germany might rise a possible threat not only...
for the adjacent roe deer population but also for sheep herds grazing on pastures close to affected forest sections (Foreyt 1990). According to reports of experimental infections of ovises with Fascioloides-metacercariae leading to fatal casualties caused by a small number of immature migrating flukes within a short period of time, it should be avoided to raise sheep in regions with evidence of the giant liver fluke (Foreyt 1996, Foreyt and Todd 1976). Thus, the frequent tracking of Fascioloides-positive wild ungulates as previously realized in Austria seems to be of major importance in order to avert possible related economic damages (Ursprung et al. 2006). Since detailed information on the current spreading of the giant liver fluke due to natural migrations of wild ruminants in northeastern Bavaria is still scarce, the present study was performed to gain data on the occurrence and geographical expansion of F. magna and other liver parasites in cloven-hoofed game from 14 counties in Upper and Middle Franconia, Upper Palatinate, and Lower Bavaria.

Methods

Sample origin

From October 2019 to February 2020, a total of 700 shot pieces of cloven-hoofed game was investigated for the presence of F. magna and accompanying liver parasites at the Bavarian Health and Food Safety Authority (LGL), Erlangen, Germany. Livers were obtained from 640 pieces of red deer (Cervus elaphus; 251 male, 389 female), 24 pieces of roe deer (Capreolus capreolus; four male, 20 female), one piece of fallow deer (Dama dama; male), and 35 pieces of wild boar (Sus scrofa; 17 male, 18 female).

The majority of samples (554/700) was collected during driven hunts at the military training grounds Grafenwöhr (counties Amberg-Sulzbach/Neustadt a.d. Waldnaab) and Hohenfels (counties Neumarkt/Amberg-Sulzbach). The second largest collection region was the Bavarian Forest National Park (counties Regen/Freyung-Grafenau) with 110 samples. The remaining livers (36/700) originated from animals shot in the framework of private hunts in eight different counties in northeastern Bavaria (Fig. 1). All samples were investigated directly within 24 to 48 h after arrival in Erlangen or frozen at −20 °C until further processing.

Pathological and histological investigations

During a first macroscopic examination, the surfaces of all livers were screened for any pathological alterations before the organs were cut into slices with a maximum thickness of 1 cm in order to evaluate the liver parenchyma in detail. Any pathological-anatomical changes of the livers were documented, and in case of a suspected infection with F. magna, a categorization of the alterations was conducted into “absent” (−), “minor” (+), “moderate” (++), or “major extent” (+++) according to Plötz et al. (2015). Altered liver tissue was cut into small pieces, fixed in 4% neutral-buffered formaldehyde for at least 24 h and processed routinely for the histological investigation. After hematoxylin-eosin (HE)-staining, the slides were examined microscopically with 25 × to 630 × magnification using a light microscope.

Parasitological investigations

All livers were screened for liver parasites macroscopically. Number and size of adult parasites or developmental parasitic stages were documented followed by a morphological species determination (Naem et al. 2012). Liver slices of altered organs were covered with water over night for a sedimentation process. After 12–24 h, the liver tissue was dumped into a sieve and rinsed with tap water. The sieve residual was examined for adult or juvenile parasitic stages. The rinsing liquid was sedimented for an hour followed by several sedimentation periods of 15 min until excess particles were removed. The final sediment was examined for parasite eggs in a petri dish with 100 × magnification using a light microscope.

Molecular investigations

Parasitic material (adults, juvenile stages, or eggs) as well as liver tissue with morphological alterations of categories (+)–(+++) was frozen at −20 °C until further investigation by PCR. Tissue samples were taken at four representative locations per F. magna-suspicious liver. DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommended protocol for tissues with initial incubation of specimens in buffer ATL and Proteinase K at 56 °C overnight. Isolated DNA was stored at −20 °C until further use. For the amplification of partial ITS-2 regions of F. magna, F. hepatica, or D. dendriticum, an individual set of specific primers was used (Králová-Hromadová et al. 2008, Bázsalovicsová et al. 2010, Table 1). Developmental stages of cestodes detected in liver material of red deer and wild boar were differentiated by multiplex PCR according to Trachsel et al. (2007), targeting the genes nad1 of E. multilocularis and rnsS of E. granulosus/Taenia spp., respectively (Table 1). PCR reactions with trematode and liver samples were performed in a total volume of 50 µl, containing 5 µl of template DNA, 0.5 µM of each primer, 1.5 mM MgCl2, 1U Invitrogen Platinum™ Taq DNA Polymerase (Thermo Fisher Scientific, Darmstadt, Germany), and 0.1 mM dNTP-Mix (Roche, Mannheim, Germany) using a BIOMETRA T3 thermocycler (Analytic Jena AG, Germany). Multiplex PCR of cestode samples was performed using the QIAGEN Multiplex PCR Kit with a
reaction volume of 50 µl, containing 25 µl Qiagen master mix, 5 µl of template DNA, and 5 µl of primer mix (2 µM of each primer Cest1-4 and 16 µM of primer Cest5; Table 1). PCR products were visualized on 1.5% agarose gels, purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations and sequenced in two directions by MWG Eurofins Genomics sequencing services (Ebersberg, Germany). Forward and reverse sequences were complemented and aligned using online tools (Reverse Complement, http://www.bioinformatics.org/sms/rev_comp.html; ClustalOmega, https://www.ebi.ac.uk/Tools/msa/clustalo). Database searches and sequence comparisons were performed with BLAST provided by the National Center for Biotechnology Information (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Fig. 1 Fourteen selected counties (marked in yellow, green, and red) for sample collection in Upper and Middle Franconia, Upper Palatinate, and Lower Bavaria, namely, Amberg-Sulzbach (AS), Bayreuth (BT), Cham (CHA), Forchheim (FO), Freyung-Grafenau (FRG), Hof (HO), Kulmbach (KU), Neustadt a. d. Waldnaab (NEW), Neumarkt (NM), Nürnberger Land (LAU), Regen (REG), Schwandorf (SAD), Tirschenreuth (TIR), and Wunsiedel (WUN). Counties marked in red: sample submissions with detection of *F. magna*; counties marked in green: sample submissions without detection of *F. magna*; counties marked in yellow: selected for sample collection, but without sample submissions (no data available). Streaked circle 1: Veldensteiner forest; streaked circles 2–4: origin of 95% of investigated samples (2 military training ground Grafenwöhr, 3 military training ground Hohenfels, 4 Bavarian Forest National Park). Inset: location of included counties in Germany.
The occurrences of *F. magna* and other liver parasites for different origins, age, and sex groups were compared with a χ²-test. *p*-values < 0.05 were considered to be significant.

### Results

#### Pathological findings

A macroscopically visible hepatic damage indicating an infection with *F. magna* was present in 38 samples originating from red deer. Mild pathological alterations of the liver tissue were documented in 15, moderate in 14, and major in nine cases, respectively (Fig. 2a–c). Livers exhibiting *Fascioloides* specimens (23 cases, Fig. 2d–e) showed black pigmented streaks as well as pseudocysts lined with connective tissue (up to 5 cm in diameter) filled with one up to 66 *F. magna* specimens (median value of 3) with a body length ranging from 1.5 to 8 cm (Fig. 2a–e). Similar hepatic alterations were present in livers without *Fascioloides* specimens except that migration tracks and pseudocysts were filled with yellowish to dark brown material of fluid, pasty, pulpy, or firm consistency.

Acute fresh infections were marked by solitary migration tracks filled with fresh blood and liquid liver tissue (Fig. 2f). In chronic cases, the entire liver was indurated with connective tissue and partly minimized in size (Fig. 2c). In 68.4% of *F. magna*-positive livers, slight to severe perihepatitis villosa was present (Fig. 2h).

#### Histological alterations in *F. magna*-positive livers

Histologically, the trematode-specific black blood pigment of hemoglobin origin “haematin” was present in 37 of 38 *Fascioloides*-suspicous livers as well as in single liver lymph nodes (Fig. 3a–d). In most cases, black pigmented migration tracks were formed within the hepatic parenchyma whereas tightly clustered pigment accumulations marked the ground part of fibrous capsules. Additionally, large thin-walled trematode eggs were found in variable quantity in livers populated with mature flukes (Fig. 3b). Further histopathological results typical for *F. magna* were moderate to high-grade periportal fibrosis, lymphoplasmacellular inflammatory reactions (Fig. 3c), focal hemorrhage, and tissue necrosis. The severity of all mentioned alterations varied depending on the infectious stage of the affected animals.

| Table 1 Species-specific primers and cycling conditions for differentiation of fluke species and cestodes by conventional PCR |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Parasite species            | Forward primer 5´-3´         | Reverse primer 5´-3´         | Amplicon size 152 bp         | Cycling conditions           |
| *Fascioloides magna*        | FM-ITS2-SPEC-F ACC AGT TAT CGT TGT GTT G | FM-ITS2-SPEC-R CCG TCT TTA AAC AAC AG | 152 bp                      | initial denaturation: 2 min 94 °C |
| *Fasciola hepatica*         | FH-ITS2-SPEC-F CTT ATG ATT TCT GGG ATA ATT | FH-ITS2-SPEC-R CCG TCG CTA TAT GAA AA | 112 bp                      | 35 cycles (F. magna) resp. 40 cycles (F. hepatica): 30 s/94 °C 30 s/46 °C 30 s/72 °C final elongation: 5 min/72 °C |
| *Dicrocoelium dendriticum*  | DD-ITS2-SPEC-F CCC CAG TCG GAA ACG TCA | DD-ITS2-SPEC-R GAT TAG AAG GCC GTA TTT CGG A | 176 bp                      | initial denaturation: 2 min 94 °C 35 cycles: 30 s/94 °C 30 s/55 °C 30 s/72 °C final elongation: 5 min/72 °C |
| *Echinococcus multilocularis* | Cest 1 TGC TGA TTT GTT AAA GTT AGT GAT C | Cest 2 CAT AAA TCA ATG GAA ACA ACA ACA AG | 395 bp                      | initial denaturation: 15 min/95 °C 40 cycles: 30 s/94 °C 30 s/85 °C 30 s/72 °C final elongation: 5 min/72 °C |
| *Echinococcus granulosus*   | Cest 4 GTT TTT GTG TGT TAC ATT AAT AAG GGT G | Cest 5 GCG GTG TGT ACM TGA GCT AAA C | 117 bp                      | 35 cycles: 90 s/72 °C 90 s/72 °C final elongation: 5 min/72 °C |
| *Taenia spec*               | Cest 3 YGA YTC TTT TTA GGG GAA GGT GTG | Cest 5 GCG GTG TGT ACM TGA GCT AAA C | 267 bp                      | 35 cycles: 90 s/72 °C 90 s/72 °C final elongation: 5 min/72 °C |
Fig. 2  a–h Pathological-anatomical alterations in *F. magna*-positive livers from red deer

a) Minor tissue alterations: single black pigmented migration streaks at the liver surface

b) Moderate tissue alterations: multifocal perihepatitis villosa, multiple black pigmented streaks of divergent size, fibrous capsule at the liver surface of the right liver lobe

c) Major tissue alterations: complete organ minimized in size and interspersed with fibrous tissue (cirrhosis), multifocal multiple black pigmented streaks of divergent size, numerous fibrous capsules at the liver surface

d) Adult specimen of *F. magna* with body length of 5.2 cm, oral sucker facing to the left
Fig. 2 (continued)

**e)** Cross section into a fibrous capsule within the liver parenchyma filled with a single *F. magna*-specimen

**f)** Cross section into multiple fresh migration tracks filled with clotted blood

**g)** Cross section into black pigmented migration track filled with yellowish detritus

**h)** Diffuse multifocal perihepatitis villosa
**Fig. 3 a–d** Common histological alterations in *F. magna*-positive livers and portal lymph nodes

**a)** Liver tissue, red deer, HE-staining, 50 × magnification: migration tracks consisting of haematin surrounded by periportal fibrosis (marked with white arrows) and inflammation reactions (marked with white stars)

**b)** Liver tissue, red deer, HE-staining, 200 × magnification: longitudinal and cross section of multiple trematode eggs as surrounded by minor clusters of haematin

**c)** Liver tissue, red deer, HE-staining, 200 × magnification: periportal lymphoplasmacellular inflammation with involvement of eosinophilic granulocytes and central hyperaemia. Additionally, circumscribed accumulation of fine haematin marks (marked with white arrows)

**d)** Liver lymph node, red deer, HE-staining, 100 × magnification: multifocal diffuse abundant deposits of haematin within the lymphatic tissue
Parasitological and molecular investigations

Mature or immature specimens of the giant liver fluke were detected and morphologically identified in 23 out of 38 Fasciolaoides-suspicous livers. Fluke material was further examined by F. magna-specific PCR (Table 1) to proof the results of the morphologically based determinations. All DNA samples produced amplicons of the expected size (152 bp) with sequence homologies between 99 and 100% to GenBank accession no. DQ683545.1 (partial F. magna 5.8S ribosomal RNA gene and internal transcribed spacer 2). Large trematode eggs of the type Fasciolaoides/Fasciola were present in 31 sediments of rinsing liquid of liver tissue (Table 2). In 21 cases, those eggs could be identified as F. magna by PCR. In two samples, it was possible to identify the eggs as Fasciola hepatica with 100% sequence homology to GenBank accession no. MT423007.1. Both cases could be classified as single infections with the latter trematode. In eight samples, the extraction of DNA from eggs was insufficient for a determination of the fluke species by PCR.

In 63 (9%) of all 700 investigated livers, other liver parasites besides F. magna were detected and further investigated by PCR for species identification. Namely, parasitic stages of Dicrocoelium dendriticum (40 pieces of red deer and two roe deer, 6%), Fasciola hepatica (two pieces of red deer, 0.3%), Taenia hydatigena (11 pieces of red deer and one wild boar, 1.7%), and Echinococcus multilocularis (7 pieces of wild boar, 1.0%) were found. Coinfections were restricted to four cases in red deer harboring F. magna and D. dendriticum simultaneously. A single coinfection with T. hydatigena and E. multilocularis occurred in the liver of one wild boar.

Occurrence of F. magna and regional distribution

In consideration of all utilized diagnostic methods (morphological identification of fluke specimens, Fasciolaoides-DNA or haematin in liver tissue), F. magna was detected in 5.9% (38/640) of livers from red deer (Table 2), whereas none of the investigated livers of wild boar, roe, and fallow deer was infected. Statistically, an upper limit for the occurrence in the investigated livers of wild boar, roe, and fallow deer was estimated as 8.2% and 11.7%, respectively (p = 0.05). Animals older than 1 year were significantly more often infected with the trematode compared to animals younger than 1 year (p < 0.05; Table 3). The difference of occurrence rates for F. magna between male and female pieces was statistically insignificant (p = 0.76).

According to the results of the present study, the American suction worm can be detected in the military training ground Grafenwöhr and in the Bavarian Forest National Park with an estimated prevalence of 3.6% and 16.4%, respectively (Table 4). A determination of the regional occurrence of F. magna for all other included districts was not applicable due to low sample numbers.

Discussion

The evidence of mild to moderate alterations in the majority of infected livers (29/38 or 76.3%) is well known for red deer belonging to the group of definitive hosts of F. magna (Pybus 2001). Equivalent lesions are caused by limited migration of immature flukes followed by subsequent encapsulation of mature specimens which is usually associated with pressure atrophy of adjacent liver parenchyma (Pybus 2001). In general, the extent of liver damage in positive animals varied depending on the amount of flukes and age of infection in the present study. Thus, a high fluke burden within one organ resulted in higher number of fibrous capsules and a chronic course of infection was marked by increased general fibrosis of liver tissue. Positive livers containing F. magna specimens harbored a median of three flukes representing a general low infection burden in the investigated animal population. In the course of its life, F. magna might grow up to the remarkable size of 8–10 cm and reaches sexual maturity with a body length of 3–5 cm leading to patent infections marked by egg-shedding with the feces of the infected host (Ursprung et al. 2006). Accordingly, trematode eggs were only diagnosed in the sediment of livers populated by flukes with a minimum size of 3 cm in the study at hand.

In most recently conducted surveys, the diagnosis of F. magna relies on a macroscopic investigation of liver tissue, solely (Novobilisky et al. 2007; Plötz et al. 2015; Rehbein 2017). In the present study, the detection of F. magna by morphological methods was confirmed by molecular analyses. This approach allows a reliable identification of the fluke species and provides a method for the species-specific discrimination of F. magna from other species of the genus Fasciola. The results of this study indicate a low prevalence of F. magna in red deer compared to wild boar and roe deer, which is consistent with previous reports from Germany and other European countries. However, the difference in occurrence rates between male and female pieces was not statistically significant. Further studies are needed to investigate the potential role of red deer in the transmission of F. magna and to assess the impact of this infection on the health of red deer populations.
et al. 2021; Ursprung et al. 2006). Furthermore, in the framework of some of those epidemiological surveys, organs were only further investigated after the detection of suspicious alterations in the affected livers by hunters. Consequently, *F. magna* possibly represents an underdiagnosed parasite since slight changes in the liver tissue might be overlooked at first sight in the field. In order to collect reliable data on the occurrence of *Fascioloides*, the inset of additional diagnostic methods on a broad set of non-preselected samples is highly recommended. Besides the general macroscopic examination, a histological investigation of affected liver tissue seems to be a valuable supplementary diagnostic method in order to verify an infestation with *F. magna* since it is not always possible to detect adult stages or trematode eggs (Table 2).

The migration of immature *Fascioloides*-flukes has been reported to be associated with the formation of characteristic haematin in hepatic tissue (Campbell 1960; Blažek and Gilka 1970). Hence, all 37 out of 38 *Fascioloides*-suspicous livers containing this pigment of hemoglobinic origin were considered as *F. magna*-positive in the framework of this study (Table 2). Thirty-three of these cases were confirmed by the detection of *Fascioloides*-DNA obtained from hepatic tissue, large trematode eggs, or fluke material. In four cases, the histological finding of haematin was the only indication for an infection with the large American liver fluke while neither gross *Fascioloides*-specific pathological alterations, nor flukes, nor trematode eggs were present. It was possible to verify two of those suspected cases by subsequent PCR.

The exclusive histological investigation might reveal false negative results in chronic cases with encapsulated flukes and sample collection at the wrong location within the liver tissue (Campbell 1960). Accordingly, no haematin was detectable in the histological cut of one liver in the present survey despite the molecular verification of infection (Table 2). The investigation for *F. magna*-like eggs in the sediment of altered liver tissue is another complementary method to confirm the diagnosis, especially in chronic infections with macerated *Fascioloides*-specimens. Even though macroscopic liver alterations differ depending on the concerned trematode species, an investigation of large trematode eggs by PCR with subsequent sequencing of obtained isolates is necessary to differentiate *F. magna* from *Fasciola hepatica* whose eggs appear identical microscopically (Bazsalovicsová et al. 2010; Králová-Hromadová et al. 2008).

The macroscopic examination of livers revealed other liver parasites besides *F. magna* in almost 9% (n=63) of all included animals. Unexpectedly, coinfections of the invasive species *F. magna* and *Fasciola hepatica* were not present even though both flukes share the same intermediate host and *F. hepatica* is a common endoparasite in cervids (Böhm et al. 2006). It is also worth mentioning that infections with *Fascioloides* were nineteen times more frequent in the investigated material than infections with *Fasciola*. This might indicate that the giant liver fluke is even better adapted to habitats, intermediate, and final hosts than its indigenous counterpart. One out of three animals shedding eggs of both *Dicrocoelium dendriticum* and *F. magna* showed a chronic cholangitis which is pathognomonic for the small liver fluke. The other two livers had lesions compatible with a *Fascioloides*-infection, confirming that *Dicrocoelium* infections are generally less apparent than those caused by other flukes.

### Table 3: Number of *F. magna*-positive livers obtained from red deer with respect to collected number of samples per age group and sex

| Age (years) | Male       | Female     | Total     |
|------------|------------|------------|-----------|
| 0–1        | 1/112 (0.9%) | 1/162 (0.6%) | 2/274 (0.7%) |
| > 1        | 14/139 (10.1%) | 22/227 (9.7%) | 36/366 (9.8%) |
| Total      | 15/251 (6.0%) | 23/389 (5.4%) | 38/640 (5.9%) |

### Table 4: Detection and regional occurrence of *F. magna* in red deer

| County/region          | Number of red deer | Detection of *F. magna* | Regional occurrence |
|------------------------|--------------------|-------------------------|---------------------|
| Neustadt a.d.W./Amberg-Sulzbach (military training ground Grafenwöhr) | 418                | 15                      | 3.6%                |
| Neumarkt/Amberg-Sulzbach (military training ground Hohenfels) | 91                  | 1                       | 1.1%                |
| Freyung-Grafenau/Regen (Bavarian Forest National Park) | 110                | 18                      | 16.4%               |
| Tirschenreuth          | 14                 | 0                       | *                   |
| Freyung-Grafenau       | 2                  | 2                       | *                   |
| Amberg-Sulzbach        | 2                  | 1                       | *                   |
| Cham                   | 1                  | 0                       | *                   |
| Schwandorf             | 1                  | 1                       | *                   |
| Nürnberger Land        | 1                  | 0                       | *                   |
| Total                  | 640                | 38                      | 5.9%                |

*The determination of a regional occurrence was not applicable due to a low sample number*
Since the first record of *F. magna* in wild ungulates in northeastern Bavaria, Germany, in 2011, the imported American trematode has successfully established further foci in parts of the Upper Palatinate and Upper Franconia (Plötz et al. 2015; König et al. 2019). Especially, the Veldensteiner forest in Upper Franconia (Upper Franconia) has recently been identified as a hot spot of the giant liver fluke colonization game, with reported local prevalence rates of 40–71% in red deer (König et al. 2019). The latter forest is located next to the military training ground Grafenwöhr, Upper Palatinate with an estimated prevalence of 3.6%. A total of 16.4% of all investigated red deer from Grafenwöhr, Upper Palatinate with an estimated prevalence of 3.6%. A total of 16.4% of all investigated red deer from the Bavarian Forest National Park were infected with *F. magna* representing the first report of *F. magna* in Lower Bavaria. A possible explanation for the effective dispersion of the parasite in Germany is the migration of infected cloven hoofed game from highly endemic regions in the Czech Republic to bordering German counties harboring suitable habitats for appropriate intermediate hosts enabling a completion of the infection cycle (Rehbein et al. 2021). Within the last decades, numerous endemic *F. magna* foci have been reported from the Czech Republic with prevalences ranging up to 95% in populations of both free and game ranging cervids (Erhardova-Kotrla 1971; Kasny et al. 2012; Novobilský et al. 2007). As early as 2007, so-called enzootic wet regions in the west of the country providing ideal living conditions not only for freshwater snails as intermediate hosts but also for deer as final hosts have already been assumed to support the spread of the giant liver fluke to adjacent regions in southeastern Germany (Novobilský et al. 2007).

Whereas the impact of the sex turned out to be insignificant, the age of *Fascioloides*-infected animals of the study was statistically significant in the present study. Accordingly, animals older than 1 year were 13 times more often infected compared to animals younger than 1 year. Those findings are in accordance with Rehbein et al. (2021) and
Plötz et al. (2015) and might be due to the wide range of variations concerning the prepatent period of the giant liver fluke in ruminant hosts ranging from three up to 7 months (Foreyt and Todd 1976). Thus, most infected animals do not show typical liver lesions in their first year of life because of the long development time of immature flukes.

To summarize the major results of the present study, the giant liver fluke should be considered as an emerging endoparasite in red deer from northeastern Bavaria. The detection of novel habitats of the fluke in Lower Bavaria raises the assumption that neighboring counties of recent Fascioloides hotspots also harbor large populations of the giant liver fluke. Since high mortality rates in connection with a so-called Fascioloides-malnutrition syndrome have been described in infected game ranging red deer, increasing numbers of affected animals should be taken seriously with regard to the preservation of a good health status of the cloven-hoofed game population in the affected counties (Balbo et al. 1989). Consequently, a continuation of the monitoring in northeastern Bavaria is advisable for the successive hunting seasons. Regarding the composition of samples, a higher number of livers originating from roe deer and sheep as endangered aberrant hosts should be included in order to get information on the spreading of F. magna through infected red deer populations. The outcome of the comparative investigations of the present study emphasizes the importance to apply different diagnostic methods besides a macroscopic examination of livers for a reliable detection of F. magna.

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Availability of data and material One gene sequence (partial ITS-2-gene) of an adult Fascioloides magna specimen which was identical with all Fascioloides-sequences obtained within the framework of the present study was uploaded exemplarily to GenBank Ac. No. OK093210.

Declarations

Conflict of interest The authors declare no competing interests.

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