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Korrespondenzadresse:  
m.scheuerle@lmu.de

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### Summary

### Zusammenfassung

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Lehrstuhl für Vergleichende Tropenmedizin und Parasitologie,  
Ludwig-Maximilians-Universität München<sup>1</sup>  
Kathrinenhof Research Center der Merial GmbH<sup>2</sup>  
Lehr-, Versuchs- und Demonstrationsanlage für landwirtschaftliche  
Gehegewildhaltung Pfrentsch<sup>3</sup>  
Tierärztliche Fachpraxis für Kleintiere<sup>4</sup>

## *Fascioloides magna* – epizootiology in a deer farm in Germany

### *Epizootologie von Fascioloides magna in einem Wildgehege in Deutschland*

Cornelia Plötz<sup>1</sup>, Steffen Rehbein<sup>2</sup>, Helmut Bamler<sup>3</sup>, Hubert Reindl<sup>4</sup>, Kurt Pfister<sup>1</sup>,  
Miriam C. Scheuerle<sup>1</sup>

After initial observations of suspicious cases in 2009, the occurrence of *Fascioloides (F.) magna* in deer of a deer farm located in northeastern Bavaria, Germany, at the border to the Czech Republic was confirmed in autumn 2011. In March 2012, the deer were treated for fascioloidosis with triclabendazole. To monitor the epizootiology of fascioloidosis in the farm, 80–100 faecal samples were examined for *Fascioloides* eggs at monthly intervals from June 2012 to June 2013 inclusive. In addition, livers of 27 red deer and one sika deer collected during winter 2012/2013 were examined for gross lesions suspicious for *F. magna* infection and 21 of the 28 livers were dissected for *F. magna* recovery. *Fascioloides* eggs were recorded in 63 (4.9%) of 1280 faecal samples (range 0.4 to 355 eggs per gram). Both, number of *Fascioloides*-egg positive samples and egg counts were low during the first eight months of the study but increased notably since February 2013. While *Fascioloides* egg-positive faecal samples were obtained from red deer (46/948, 4.9%) and fallow deer (17/166, 10.2%), no *Fascioloides* eggs were demonstrated in the 166 samples obtained from sika deer. Livers of five red deer and the sika deer showed gross lesions characteristic for fascioloidosis, and *F. magna* were recovered from three of the five affected red deer livers (range, five to seven flukes). Results of this study confirm that *F. magna* is endemic in the deer farm, and measures should be implemented to minimize the transmission of the parasite.

**Keywords:** giant liver fluke, Bavaria, spread, triclabendazole, deer

Nach Registrierung erster verdächtiger Fälle im Jahr 2009 wurde im Herbst 2011 das Auftreten von *Fascioloides (F.) magna* in einem in Nordostbayern, Deutschland, an der Grenze zur Tschechischen Republik gelegenen Wildgehege bestätigt. Im März 2012 wurde das Wild im Gehege mit Triclabendazol gegen Faszioleiose behandelt. Um die Epizootiologie der Parasitose zu verfolgen, wurden von Juni 2012 bis einschließlich Juni 2013 monatlich 80 bis 100 Losungsproben auf *Fascioloides*-Eier untersucht. Zusätzlich wurden die Lebern von 27 Stück Rotwild und einem Stück Sikawild, die im Winter 2012/2013 erlegt wurden, auf für *F. magna*-Infektionen typische pathologisch-anatomische Veränderungen untersucht und 21 dieser Lebern einer Sektion unterzogen. In 63 (4,9 %) von 1280 Losungsproben wurden *Fascioloides*-Eier nachgewiesen (0,4–355 EpG). Sowohl die Anzahl der Proben, in denen *Fascioloides*-Eier gefunden wurden als auch die darin ermittelten Eizahlen waren während der ersten acht Monate der Studie niedrig, stiegen aber seit Februar 2013 deutlich an. Während *Fascioloides*-Eier sowohl in den Losungsproben von Rotwild (46/948; 4,9 %) als auch von Damwild (17/166; 10,2 %) nachgewiesen wurden, war keine der 166 Proben des Sikawildes *Fascioloides*-positiv. Fünf der seziierten Rotwildlebern, sowie die Leber des Sikawildes wiesen für Faszioleiose typische Veränderungen auf, wobei in drei dieser Rotwild-Lebern *F. magna*-Exemplare (fünf bis sieben Egel pro Leber) nachgewiesen wurden. Die Ergebnisse dieser Studie bestätigten, dass das Auftreten von *F. magna* in diesem Gehege als endemisch zu betrachten ist. Es sollten Maßnahmen getroffen werden, um die Übertragung des Parasiten einzuschränken.

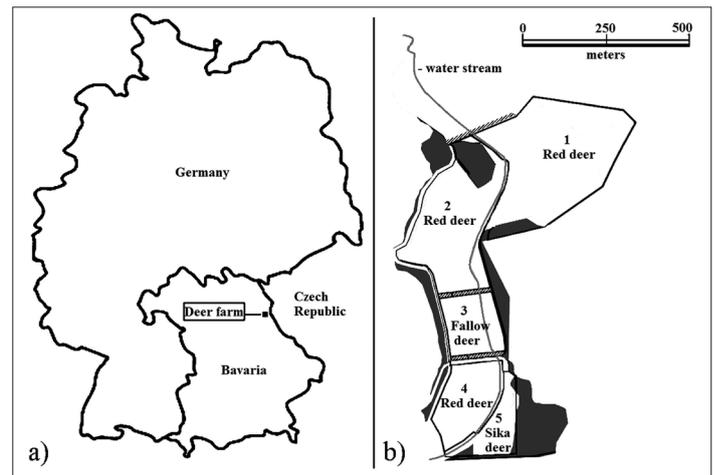
**Schlüsselwörter:** Amerikanischer Riesenleberegel, Bayern, Ausbreitung, Triclabendazol, Wild

## Introduction

The giant liver fluke, *Fascioloides* (*F.*) *magna* (Bassi, 1875), is considered as a highly pathogenic, invasive liver fluke affecting ungulates in central Europe. Originally a common, relatively well-tolerated parasite of native North-American cervids (e. g. white-tailed deer, wapiti), it has been introduced to continental Europe accidentally with naturally infected deer imported on several occasions in the 19<sup>th</sup> and 20<sup>th</sup> century (Erhardová-Kotrlá, 1971). *F. magna* successfully adapted its life-cycle, which resembles the one of *Fasciola hepatica* in many aspects, including the main intermediate host *Galba truncatula*, to conditions in Europe (Ursprung et al., 2006). While not spreading from the location of its first detection in Northern Italy, *F. magna* spread from the places of its introduction in central Europe, i. e. Bohemia and Moravia, especially in the past two decades: spreading occurred initially mainly in eastern direction with reaching the Danube floodplain system in Slovakia in the early 1990s (Králová-Hromadová et al., 2011; Špakulová et al., 1997). Further dispersal along the Danube led to the finding of *F. magna* in deer in Austria, Hungary, Croatia and Serbia (Erhardová-Kotrlá, 1971; Janicki et al., 2005; Králová-Hromadová et al., 2011; Sattmann et al., 2014; Špakulová et al., 2003). Recent surveys in the Czech Republic revealed a westward spreading of the parasite from its historical endemic areas in Central and South Bohemia (Kašný et al., 2012; Novobilský et al., 2007). Consequently, the ‘arrival’ of *F. magna* in northeastern Bavaria, Germany, next to the border to the Czech Republic, was reported in 2012 (Rehbein et al., 2012). Questioning of hunters in the area where *F. magna* was recorded in Bavaria revealed that deer showing hepatic lesions suspicious for giant liver fluke infection were observed probably as early as 2007. Previously, *F. magna* was recorded in red deer in Lower Silesia, Germany (now part of Poland) in 1930 (Salomon, 1932) and reported thereafter from that locality only once (Ślusarski, 1955, 1956) before being ‘rediscovered’ in the area in 2013 (Demiaszkiewicz, 2014).

At the end of the year 2009, first suspicious liver lesions were noticed in the deer in a deer farm (Pfrentsch) located in the same area. As frequency and severity of the liver lesions increased over time in both free-living and farmed deer, one altered liver was submitted to the Diagnostic Center of the chair of Comparative Tropical Medicine and Parasitology, LMU Munich, for laboratory examination in October 2011, and the suspected diagnosis of fascioloidosis was confirmed. Preliminary investigations of livers of free-living red deer, sika deer and roe deer, and farmed red deer originating from the area from November 2011 through January 2012 revealed a high prevalence of *F. magna* (7/10, 3/8, 1/5, and 8/14 deer, respectively; Rehbein et al., 2012). After diagnosis, the deer of the farm Pfrentsch were treated against fascioloidosis in March 2012 using medicated supplemental feed to provide triclabendazole (FASINEX®, Novartis), at the dose of 10 mg per kg body weight per day for five consecutive days as described previously (Ursprung et al., 2006).

The aim of the present study was to monitor the epizootiology of fascioloidosis in the deer farm after flukicide treatment by monthly examination of faecal samples for *Fascioloides* eggs and examination of livers of harvested deer for *F. magna*.



**FIGURE 1:** Map of Germany, indicating the location of the deer farm Pfrentsch in Germany (a) and sketch of the deer farm with Fields 1 to 5 (b).

## Material and Methods

### Description of the deer farm

The deer farm Pfrentsch was established in 2004 on arable and grass land that had been used for feed production before. It belongs to the Bavarian State Institute of Agriculture (Bayerische Landesanstalt für Landwirtschaft, LfL) and serves for teaching and demonstration of deer farming. The deer farm is located nearby the village Pfrentschweiher in the northeast of the district Oberpfalz, Bavaria (Fig. 1a) next to the border to the Czech Republic. The initial breeding stock (red deer, *Cervus elaphus*; fallow deer, *Dama dama*; sika deer, *Cervus nippon*) was obtained from several deer farms in Germany and one deer farm in Austria. The deer farm comprises 43 ha in total and is divided into five fenced fields housing one herd of deer each (Fig. 1b). The five fields feature mainly grass land with wet patches, bushes and some woods, access to a water stream (Rehlingsbach) and one shelter each. Each deer herd consists of calves/fawns, yearlings, hinds and one to three adult stags. Supplementary feed (hay, concentrates) is provided on a need basis only. Salt licks are available all the time.

### Coprosopic monitoring

Faecal samples were collected from June 2012 to June 2013: individual (separate) fresh droppings were picked up from the ground in all five fields at monthly intervals and stored at 8–10°C until processed within five days after collection. From each thoroughly mixed faecal sample, a subsample of 5 g was analysed using a quantitative sedimentation technique (Bauer, 2006) employing a sedimentation time of 15 minutes. Finally, eggs per gram (EPG) counts were calculated. The number of faecal samples collected per field was related to the stocking in spring 2012 (Tab. 1).

### Examination of livers

Livers of 28 deer harvested between November 2012 and January 2013 were examined. All livers were grossly inspected for lesions visible on the organ's surface and palpated, and 21 livers were made available for dissection (Tab. 2). For close examination, those livers were cut into slices (appr. 1 cm) while putting gentle pressure

on the organ. Afterwards, the slices were placed in a tub with tap water overnight and the other day, liver slices and soak were poured over a sieve and rinsed with additional tap water. The sieve residual was checked for *F. magna* specimens and the rinsing liquid was examined for *Fascioloides* eggs following sedimentation. Pathologic alterations like perihepatitis, black pigmented streaks and/or migratory tracks were recorded and categorized as absent or as having a minor, moderate or major extent

(i. e. –, +, ++ or +++). The flukes were counted, measured and preserved. Additionally, a rectum faecal sample was taken from each animal and analysed as described before.

## Results

### Coproscopy

*F. magna* eggs were detected in 63 (4.9%) of the 1280 dropping samples examined in the deer farm from June 2012 to July 2013 inclusive. Field 4 (red deer) provided the largest portion of positive samples (almost 56%), followed by fallow deer Field 3 (27%) and red deer Fields 1 and 2 (combined approximately 17%); no sample collected in Field 5 housing the sika deer tested positive for *Fascioloides* eggs. The number of eggs in the positive samples varied from 0.4 to 355 eggs per gram, EPG (mean: 27.4 EPG; median: 8 EPG).

Frequency and intensity of *Fascioloides* egg-shedding were very low for almost eight months after initiation of the study in June 2012 (approximately three months following triclabendazole treatment of the deer in March). However, the number of positive samples and the EPG values began to rise towards the end of the year 2012.

Combining the results of the examination of all samples per occasion, a marked increase in both number of positive samples and mean EPG was observed since February 2013, reaching maximum values in late spring 2013 (Fig. 2). Results obtained from the four fields which provided *Fascioloides* egg-positive samples are shown in Figure 3. From the faecal samples examined per field, 21%, 10%, 2% and 1% of the samples tested *Fascioloides* egg-positive in Fields 4, 3, 2 and 1, respectively. The highest individual faecal egg counts were observed in Fields 3 and 4 (355 and 118 EPG, respectively).

Comparing the three cervid species regarding the rate of *Fascioloides* egg-positive dropping samples, positive samples were obtained more frequently from fallow deer (17/166, 10.2%) than from red deer (46/948, 4.9%), while no *Fascioloides* eggs were identified in any of the 166 faecal samples collected in the sika deer field.

**TABLE 1:** Species of deer, stocking rate and number of faecal samples collected per month

Fields	Species of deer	Stocking (number of animals excluding calves/fawn)	Number of faecal samples collected per month <sup>1</sup>
Field 1, 17 ha	Red deer	55	36
Field 2, 12 ha	Red deer	45	25
Field 3, 6 ha	Fallow deer	20	13
Field 4, 5 ha	Red deer	20	13
Field 5, 3 ha	Sika deer	20	13
Total, 43 ha		160	100

<sup>1</sup> In June 2012, 30, 20, 10, 10 and 10 samples (total 80) were collected in Field No. 1, 2, 3, 4 and 5, respectively

**TABLE 2:** Results of the examination of the livers collected from 28 animals of the deer farm from November 2012 to January 2013

Animal No.	Field	Species of deer	~Age	Body weight (kg)	Gender	Liver alterations <sup>1</sup>	Number and length of <i>F. magna</i> recovered by dissection
1	4	Red deer	5 months	70	Male	++	6 flukes in migratory tracks (length, 2–4 cm)
2	4	Red deer	5 months	55	Male	++	7 flukes in migratory tracks (length, 2–5 cm)
3	2	Red deer	5 months	60	Female	–	0
4	2	Red deer	5 months	60	Male	–	0
5	1	Red deer	5 months	60	Male	–	0
6	1	Red deer	5 months	60	Male	–	0
7	1	Red deer	10–15 years	100	Female	++	0
8	1	Red deer	6 months	80	Male	–	0
9	1	Red deer	6 months	72	Male	– <sup>2</sup>	Not applicable
10	2	Red deer	6 months	82	Male	– <sup>2</sup>	Not applicable
11	2	Red deer	6 months	58	Female	– <sup>2</sup>	Not applicable
12	1	Red deer	6 months	80	Male	– <sup>2</sup>	Not applicable
13	1	Red deer	6 months	50	Male	– <sup>2</sup>	Not applicable
14	5	Sika deer	18 months	44	Male	+++	0
15	2	Red deer	6 months	60	Female	– <sup>2</sup>	Not applicable
16	2	Red deer	6 months	60	Male	– <sup>2</sup>	Not applicable
17	1	Red deer	7 months	40	Female	–	0
18	1	Red deer	7 months	65	Male	–	0
19	1	Red deer	7 months	60	Female	–	0
20	4	Red deer	7 months	52	Female	+++	2 flukes in 1 pseudocyst (length, 6 and 8 cm) three flukes in migratory tracks (length, 2–4 cm)
21	4	Red deer	7 months	58	Female	–	0
22	2	Red deer	7 months	56	Male	–	0
23	2	Red deer	7 months	57	Male	–	0
24	2	Red deer	15–20 years	68	Female	–	0
25	1	Red deer	7 months	70	Female	+	0
26	1	Red deer	7 months	65	Male	–	0
27	1	Red deer	7 months	73	Male	–	0
28	1	Red deer	7 months	66	Male	–	0

<sup>1</sup> Liver alterations absent (–); liver alterations of minor (+), moderate (++) or large (+++) extent.

<sup>2</sup> Liver not dissected.

### Examination of 28 livers

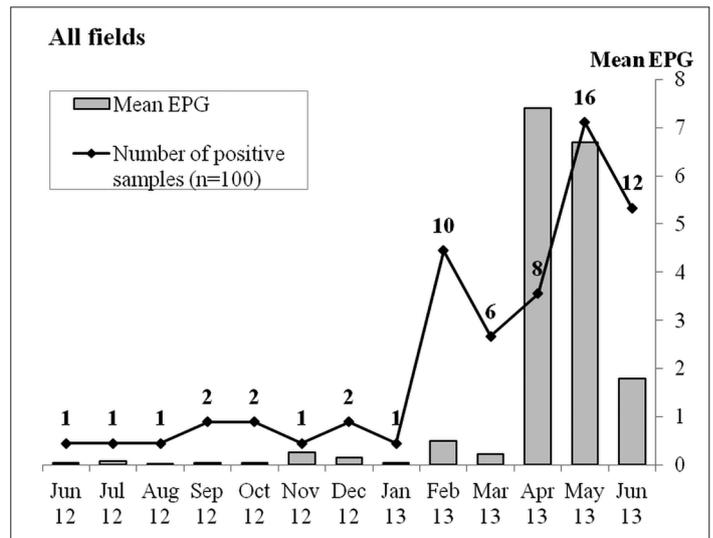
The results of the examination of the livers of 28 deer are shown in Table 2.

Gross inspection and palpation of 22 livers did not reveal any alteration, and no pathologic changes suspicious for infection of liver flukes were observed in 15 of the 21 livers which were examined by dissection. However, the livers of six deer showed lesions of variable extent on the surface and in the parenchyma which were suspicious for an infection with liver flukes (Tab. 2, highlighted in bold font). All six livers had an irregular surface with black patches and were traversed by black pigmented migratory tracks. Their pattern was not related to the liver architecture. Despite showing hepatic alterations of minor, moderate or large extent, no liver flukes were recovered from three livers (Animals 7, 14 and 25). However, fibrinous tags covered the serosa of the highly affected liver of the sika yearling (Animal 14) which was in poor general condition and carried a massive infestation with lice (*Damalinea meyeri*). Five to seven *F. magna* were recovered from the three other livers with lesions (Animals 1, 2 and 20). In the liver of Animal 20 two adult flukes were found encapsulated in a thin-walled capsule (pseudocyst) containing a reddish-black pigmented liquid. Multiple migratory tracks traversing the parenchyma of this liver were filled with the same liquid and contained three more flukes that were not yet fully grown. Compared to the liver of Animal 20, livers of both, Animals 1 (Fig. 4) and 2 showed similar but less extended alterations of the parenchyma, and the *F. magna* specimens were isolated from fresh migratory tracks only. No trematode eggs were found in the livers' rinsing liquids and rectal faeces of 21 animals but examination of the rinsing fluid of the liver and the rectum faeces of Animal 20 revealed the presence of *F. magna* eggs (7.6 EPG in faeces).

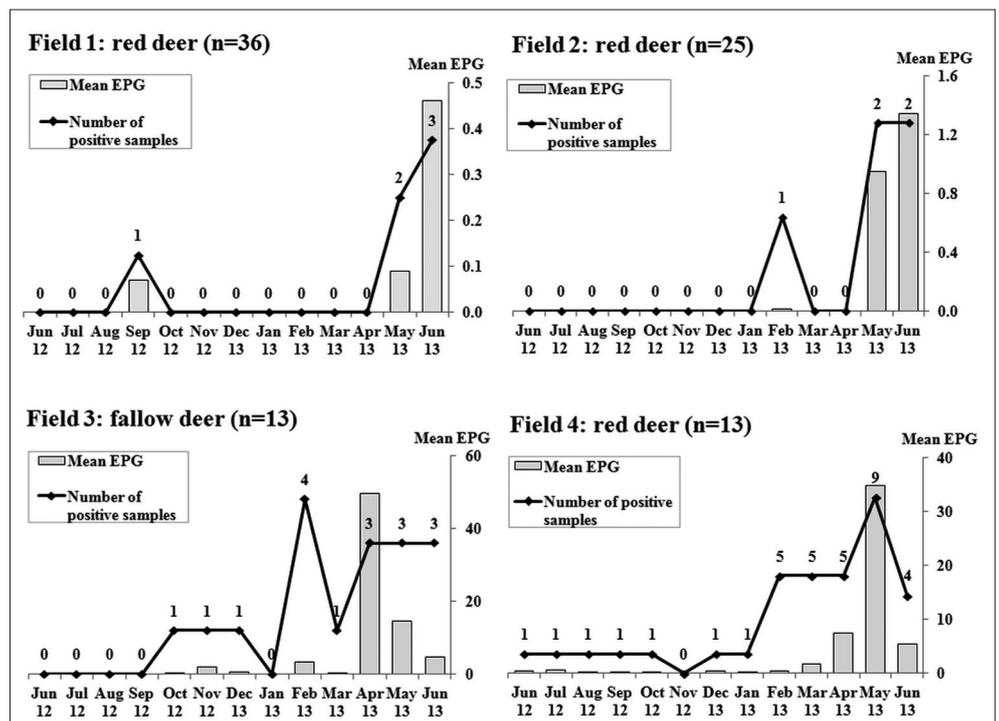
### Discussion

Monthly coproscopic examinations and the dissection of livers including the consideration of the farm's history and its location next to the German/Czech border, where *F. magna* has been diagnosed just before in free-living deer (Rehbein et al., 2012), confirmed that fascioloidosis is endemic in the deer farm Pfrentsch. The pathology recorded in six of 28 livers examined in this study matched the description of infection with *F. magna* of cervids as described previously (Erhardová-Kotrlá, 1971; Pybus, 2001).

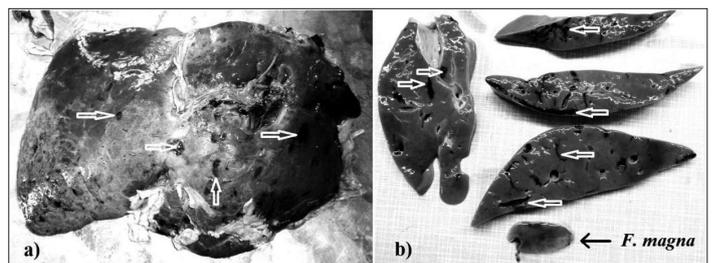
Most likely, *F. magna* was introduced into Bavaria by natural migration of infected deer from the Czech Republic, where the fluke is endemic in several areas in central and southern parts of the country. The pre-



**FIGURE 2:** Monthly number of *Fascioloides* egg-positive samples and mean faecal *Fascioloides* eggs per gram count (Mean EPG) – summary of all fields.



**FIGURE 3:** Monthly number of *Fascioloides* egg-positive samples and mean faecal *Fascioloides* eggs per gram count (Mean EPG) per field ( $n$  = number of samples collected per month).



**FIGURE 4:** Liver of Animal No. 1 (red deer calf) showing characteristic lesions of fascioloidosis: black pigmented migratory tracks (white arrows) visible from the outside (a) and deep in the parenchyma together with a juvenile fluke (length, ~3 cm) (b).

valence of the parasite in red deer populations in the Southwest of the Czech Republic varies between 4% and 95% (Novobilský et al., 2007). Recently conducted surveys recognised several new *F. magna*-positive foci in the Czech Republic which were located outside of the previously known endemic areas (Kašný et al., 2012; Novobilský et al., 2007). Some of these foci are located very close to the German/Czech border in the Šumava (Bohemian Forest), thus demonstrating the westward spreading of the giant liver fluke in the Czech Republic. Natural migration of red deer was discussed as a reason for the dispersal of the parasite in the country (Novobilský et al., 2007) where populations of red deer, fallow deer, roe deer and sika deer have shown a steady increase in both numbers and geographical distribution over the last 40 years (Bartoš et al., 2010). Furthermore, the German/Czech border does no longer create a barrier for the migration of wildlife (the border fence was removed after the fall of the 'Iron Curtain', in the Pfrementsch area from 1992 on) as confirmed through recently conducted studies monitoring the migration of both roe deer and red deer in the neighbouring Bavarian Forest/Šumava (Bohemian Forest) (Fietz and Heurich, 2004; Heurich, 2010). The spreading of fascioloidosis into Germany was therefore anticipated by Czech researchers (Kašný et al., 2012; Novobilský et al., 2007). The introduction of the giant liver fluke into the deer farm with infected breeding animals at the time of its establishment is unlikely, as the breeding stock did not originate from *F. magna* endemic areas or areas at risk of *F. magna* infection. Therefore, introduction via naturally migrating, infected deer from the Czech Republic is the most probable origin of infection of the deer in the deer farm. It is, however, unclear, whether the deer farm was built up in 2004 on ground already contaminated with infectious metacercariae following shedding *Fascioloidea* eggs by free-ranging cervids which allowed for the infection of lymnaeid snail hosts or whether infection of the deer was transmitted through passive transportation of free stages of the parasite (eggs, miracidia, cercariae, metacercariae) and/or infected intermediate snail hosts by the water of the small stream that passes all fields of the deer farm (Fig. 1b). The site's natural configuration including stable hydrological conditions through the water stream provide suitable conditions for the establishment of the giant liver fluke's life cycle. In addition, the water stream, which can be freely accessed by wild deer outside the farm, allows for a potentially steady re-contamination of the farm's fields.

Despite demonstrating success in the control of fascioloidosis in Europe by offering medicated feed in the wild in treatment programmes (e. g., Rajský et al., 2002; Špakulová et al., 2003; Ursprung et al., 2006), there are reasonable doubts that medication can sustainably decrease the giant liver fluke's prevalence and spread in free-living deer (Haider et al., 2012; Sattmann et al., 2014). Thus, the establishment of consequent and sustainable monitoring programmes and the development of well-adjusted treatment programmes in co-operation with neighbouring countries are highly recommended by researchers of affected European countries (e. g., Giczi and Egri, 2006; Kašný et al., 2012; Pybus, 2001; Rajský et al., 2002; Sattmann et al., 2014).

However, conditions are different in fenced deer: high animal densities facilitate the accumulation of infectious parasitic stages and may result in high prevalences of

infection, loss of productivity and even outbreak of fatal diseases (Balbo et al., 1989; Špakulová et al., 2003; Kašný et al., 2012). On the other hand, keeping deer in farms or enclosures allows for the management of parasitoses similar to other food animals (i. e., monitoring of drug intake, compliance with regulations regarding food safety, group treatment, separation and handling of individual animals, if necessary). Several flukicides have been tested for use of treatment of fascioloidosis in cervids but no treatment eliminated 100% of the flukes from the definitive host (for review see Pybus, 2001; Špakulová et al., 2003; Ursprung et al., 2006). However, in Croatia, where *F. magna* was detected in farmed deer and free-living deer, Janicki et al. (2005) showed that herd and individual treatment in enclosures was the most effective mode of anthelmintic therapy. As expected, the single treatment of the deer in the deer farm Pfrementsch in March 2012 was not 100% efficacious. However, although there was no pre-treatment evaluation, the very low frequency of egg-positive samples recorded until the end of the year 2012 and the lower rate of affected livers in the animals harvested in November 2012/January 2013 (6/28) compared to the results of the examination of livers one year before (8/14; Rehbein et al., 2012) are indicative of an efficacious treatment. As the treatment of the deer does not affect the parasite stages in the environment and risk of infection of final hosts is highest in late summer and fall, incidence of infection in terms of *Fascioloidea* egg-positive samples increased and varied between 6% and 16% from January 2013 on, suggesting a rise of patent infections and contamination of the fields. In a severely affected fenced herd of fallow deer in Central Bohemia, 83% of the faecal samples contained *Fascioloidea* eggs and all dissected livers were positive for flukes or typical pathology (Hirtová et al., 2003). Once the infection cycle is established in a habitat, fascioloidosis may hardly be controlled, even under farm conditions, if transmission of infection from the surrounding environment cannot be prevented (Kašný et al., 2012). This may also be the case for the deer farm Pfrementsch. To limit the impact of fascioloidosis with respect to the health and welfare of the animals but also economic losses, it is recommended to establish a once-yearly treatment as practiced. Treatment should be conducted early in the year when the deer accepts supplemental feed and thus contamination of the pastures should be reduced substantially. In addition, farm management should consider continuing parasitological monitoring and epidemiological prevention measures, e. g., restriction of the deer access to areas of high risk of infection by fencing out the water stream and moist patches in the fields.

## Conflict of interest

The authors declare that they have no conflict of interest.

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**Address for correspondence:**

Dr. Miriam Scheuerle  
 Lehrstuhl für Vergleichende Tropenmedizin und  
 Parasitologie, Veterinärwissenschaftliches Department  
 Ludwig-Maximilians-Universität  
 Leopoldstraße 5  
 80802 München  
 Germany  
 m.scheuerle@lmu.de