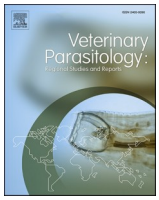
Veterinary Parasitology: Regional Studies and Reports 63 (2025) 101311

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr



Case Report

Fascioloides magna infection in a captive impala (*Aepyceros melampus*) in Florida, USA

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ARTICLE INFO

Keywords:
Fascioloides magna
Impala
Aepyceros melampus
Exotic ungulates
Trematode parasite
Liver fluke

ABSTRACT

Parasitic diseases are associated with diverse clinical signs, and veterinary professionals must be familiar with the presentations of infections in a variety of hosts. As human activity introduces new and exotic host species, expanding our understanding of pathogen effects in new species becomes integral to effective surveillance, diagnosis, and treatment. This article presents the expansion of the known host range of the American liver fluke, *Fascioloides magna*, with the infection of a farmed impala (*Aepyceros melampus*) in Marion County, Florida, USA. The gross and histopathological lesions observed are consistent with previous reports of dead-end host infections, particularly black striping across the peritoneum and abdominal viscera, a prominent pseudocyst on the visceral face of the liver, and hepatic fibrosis and biliary hyperplasia associated with tissue taken from that pseudocyst. While no whole or partial fluke was obtained from a field necropsy of the impala, amplification via cPCR and Sanger sequencing of the ITS2 region confirmed the presence of *Fascioloides magna* DNA in a frozen liver sample. This diagnostic approach was selected following the determination of an absence of fluke eggs in feces, as is characteristic of dead-end and aberrant host infections. While impala likely play little role in determining population-scale epidemiological and ecological dynamics of fluke infections, given their dead-end host status, this report serves as an important reference for managers and veterinary professionals hoping to preserve the health and welfare of exotic bovids that interface with native American cervids, the definitive hosts of *Fascioloides magna*.

1. Introduction

Parasitic infections can impact the health and production of livestock and game animals (Charlier et al., 2014; Corwin, 1997; Shanebeck et al., 2022). Parasites may be specialists or generalists, and an infection by the same pathogen may have profoundly different consequences for different species or even different individuals of the same species (Woolhouse et al., 2001). Spillover of parasites from one species of game animal to another has been documented on several occasions, with a relevant example being the discovery of the giant liver fluke, *Fascioloides magna*, in European cervids (Králová-Hromadová et al., 2011).

The liver fluke (Class Trematoda) is one of the most common parasites of farmed ungulates (Malcicka, 2015). Three species are of particular consequence to ungulate health: *Fasciola gigantica, Fasciola hepatica,* and *Fascioloides magna*. *Fasciola hepatica* is the most widespread geographically and has the largest number of definitive hosts of these three pathogens, while *Fasciola gigantica* is restricted to tropical regions (i.e., Africa and Asia) and *Fascioloides magna* to temperate regions (i.e., North American and Europe) (Mas-Coma et al., 2005). *F. magna* rarely causes disease in cervid hosts (Malcicka, 2015), but hosts outside the family Cervidae seem more affected by infection (Konjević et al., 2017; Lee et al., 2016).

All three of these trematodes pass through intermediate hosts, pond snails (Family Lymnaeidae), before encysting on vegetation to be consumed by herbivorous ungulate hosts (Malcicka, 2015). Final hosts of *F. magna* are often divided into three major categories (Malcicka,

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https://doi.org/10.1016/j.vprsr.2025.101311
Received 8 April 2025; Received in revised form 5 June 2025; Accepted 2 July 2025
Available online 16 July 2025

2015). Definitive hosts support the typical reproductive cycle of the parasite, and all known hosts in this category are in the family Cervidae. Aberrant hosts do not support the maturation and reproduction of liver flukes, and immature flukes may "wander" aimlessly throughout the body cavity. Notably, the aberrant host immune system does not allow for the formation of a capsule in the liver parenchyma surrounding flukes, a process that facilitates mating and the passing of eggs through the bile ducts and into the feces. By contrast, in a dead-end host, the immune system aids in the production of closed capsules in the liver, and eggs are not able to pass into the feces. Given the variability of *F. magna*'s consequences for different hosts, further exploration of its ecological niche and management implications is desired.

To the best of the authors' knowledge, this study presents the first report of *F. magna* infection in an impala (*Aepyceros melampus*) and applies an established molecular identification protocol to a new specimen type. The findings provide a diagnostic and pathological reference point for wildlife veterinary professionals. These data expand the known host range of *F. magna*, deepen our understanding of infection presentation in the family Bovidae, and emphasize the pathogen's relevance to the specific context of American game farms raising exotic ungulates.

2. Materials and methods

A five-year-old male impala was reported dead by a client to the University of Florida (UF)'s Cervidae Health Research Initiative (CHeRI), a free necropsy and diagnostic service available to Florida deer farmers. According to the client, the animal died at 8:00 AM on January 19, 2023. The carcass was kept in a walk-in cooler at the farm in the time between death and necropsy. A field necropsy was performed by a technician the following day at 11:00 AM. The farm and preserve at which the animal was necropsied raises white-tailed deer alongside impala and other exotic game species. No history of *Fascioloides magna* infection was noted at the location. The client described the animal as having an abnormal, hunched posture for a few days prior to death and blood emanating from the nose when it was found dead.

At necropsy, specimens were collected from the heart, kidneys, liver, lungs, and spleen. Each tissue type was cut into ~1 cm^3 sections and divided into two sets: one set was placed into 5 mL sterile Eppendorf snap-cap tubes (Thermo Fisher Scientific, Waltham, MA, USA) for fresh storage, and the other was preserved in 10 % neutral buffered formalin for histopathological evaluation. Fresh tissues were kept on ice during transport and, upon arrival at the UF College of Veterinary Medicine (CVM), stored at −80 °C unless designated for immediate bacterial culture or parasitology testing.

Fresh kidney, liver, and lung specimens were submitted to the UF CVM Microbiology, Parasitology, and Serology Diagnostic Laboratory for aerobic bacterial culture. These specimens were inoculated onto chocolate agar, Columbia Nalidixic Acid (CNA) agar, general blood agar, and MacConkey agar, and incubated under appropriate conditions to facilitate the growth of aerobic organisms. Additionally, a fecal sample was submitted to the UF CVM Diagnostic Parasitology Laboratory for centrifugal flotation, sedimentation, and McMaster egg quantification. RNA was extracted from a fresh spleen specimen using the QIAamp Viral RNA Mini kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol and tested for epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) using RT-qPCR as previously described (Cottingham et al., 2021). Formalin-fixed specimens from the lungs, heart, spleen, kidneys, and liver were submitted to the UF CVM Aquatic, Amphibian, and Reptile Pathology Laboratory for histopathological examination.

A conventional PCR assay was used to identify potential trematode infection. DNA was extracted from fresh lung and liver samples from the animal using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol. Tissue samples from another animal, a blackbuck (*Antilope cervicapra*) that died at the same location, was also tested. Extracted DNA concentrations and purities were assessed using a Nanodrop 8000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA), and samples were stored at −20 °C. The PCR reaction was performed according to protocols described by Lotfy et al. (2010) and Popovici et al. (2024) with few modifications. The PCR reaction targeted the ITS2 region, which is known to be conserved across trematodes and has been validated as useful in identifying fluke species and their phylogenetic relationships. The protocol employed forward GA1 (5′-AGA ACA TCG ACA TCT TGA AC-3′) and reverse BD2 (5′-TAT GCT TAA ATT CAG CGG GT-3′) primers. The expected amplicon size was ~500 bp.

The total reaction mixture was 25 μL, and consisted of 12.5 μL PCR Mastermix (Promega Corporation, Fitchburg, WI, USA), 1 μL each of the forward and reverse primers (diluted to 10 μL), 9.5 μL RNase-free water, and 1 μL of extracted DNA (up to 500 ng). The thermal cycling program consisted of a preliminary denaturation at 95 °C for 1 min; 32 cycles of denaturation at 95 °C for 30 s, annealing at 49 °C for 30 s, then elongation at 72 °C for 30 s; and a final elongation at 72 °C for 10 min. The reaction was held at 4 °C until the PCR tubes were removed from a SimpliAmp thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA).

The reaction product was visualized by electrophoresis in 1 % agarose gel stained using ethidium bromide. The electrophoresis was performed over the course of 60 min at 120 V and 90 mA. Migrated DNA bands were imaged using an ENDURO GDS TOUCH gel documentation system (Labnet International, Inc., Edison, NJ, USA). The PCR product was purified following visualization using a QIAquick PCR Purification Kit (QIAGEN, Valencia, CA, USA). The purified product was then submitted to Functional Biosciences (Madison, WI, USA) for Sanger sequencing in both forward and reverse directions alongside aliquots of the primers at 10 μM. Sequences obtained from the amplified ITS2 region were assembled and compared to Trematoda sequences available in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool for Nucleotides (BLASTN) (available at https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed March 27, 2025).

3. Results

Upon necropsy, the thoracic cavity was filled with blood, and black striping was present throughout the peritoneum and across the abdominal organs. Small, white nodular masses (~0.3 mm in diameter) were observed on the exteriors of the kidneys, liver, and rumen. A large circular lesion, presumably a fluke capsule, was found on the visceral face of the liver, though no fluke was found within it (Fig. 1A and B). The exterior of the liver exhibited the black striping described above and was otherwise pale grey in color. Discoloration and apparent hemorrhage were observed across the lungs (Fig. 1C and D), which may signify damage from ectopic liver flukes.

A spleen sample collected from the animal tested negative via RT-qPCR for both EHDV and BTV. Aerobic cultures of fresh samples from the lungs and kidneys produced no bacterial growth. An aerobic culture of fresh liver tissue produced scant growth of *Streptococcus gallolyticus*, which was not thought implicated in the animal's death and likely represented post-mortem growth. No fluke ova were recovered by coprological examination; however, trichostrongyle nematode ova (450 EPG) were present.

Histopathological examination revealed multifocal, chronic-active hepatitis with associated portal fibrosis and edema, bridging fibrosis, and biliary hyperplasia. Hepatitis was lymphocytic and neutrophilic. Regions of fibrosis were largely devoid of portal triads, occasionally mildly edematous, and contained small numbers of scattered neutrophils, lymphocytes, and macrophages containing a golden brown to dark brown pigment. Multifocal to coalescing hemorrhage was observed in the kidneys (mild), lungs (moderate), and liver (marked). A specific cause for these observations was not histologically evident, producing the need for further testing.

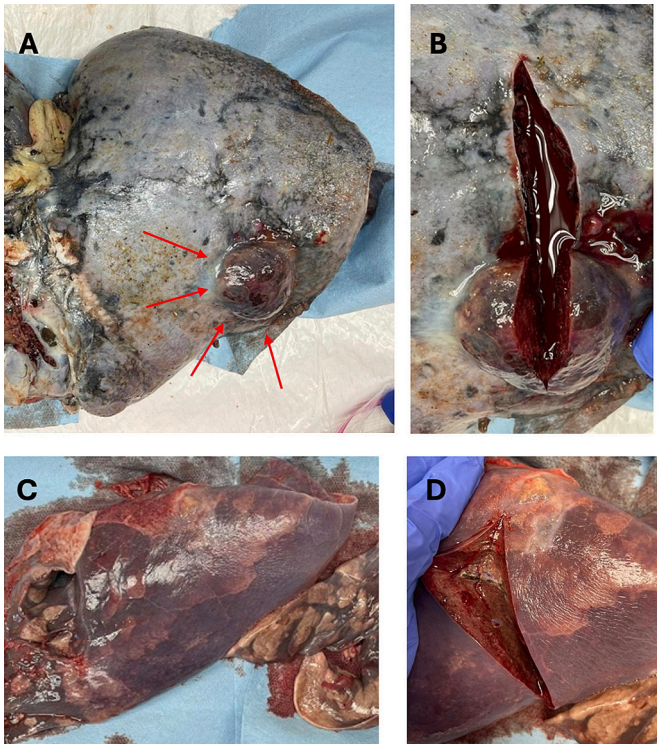

Fig. 1. Gross pathological lesions associated with *F. magna* infection of impala (*Aepyceros melampus*). The liver (1 A, 1B) displayed a pathognomic black coloration that was spread across all the abdominal viscera. The lungs (1C, 1D) showed significant discoloration and hemorrhage that may signify damage from ectopic liver flukes. A large pseudocyst (arrows, 1 A) that contained no whole or partial fluke body was found on the visceral face of liver.

Electrophoresis of the cPCR product revealed a clear, single band at approximately 500 bp (Fig. 2). Following quality trimming at a quality score (QS) of 20 and assembly, the sequenced ITS2 segment isolated from the fresh liver sample yielded a 355 bp sequence. A standard nucleotide–nucleotide BLAST (BLASTN) analysis demonstrated 100 % query coverage and 99.48 % sequence identity to *Fascioloides magna* (accession numbers EF534992.1 and DQ683545.1) in the NCBI GenBank database.

4. Discussion

This case represents the first reported incidence of a fatal fluke infection of any kind in an impala, and the first overall incidence of *F. magna* in an impala. *Fasciola gigantica* is the only fluke species previously identified in impala, and information regarding pathological changes and disease burden of those flukes in impala is limited (Malatji et al., 2020; Phiri et al., 2017). There is a report of *F. magna* in Brahman heifer imported to South Africa, part of the impala's native range, from the United States, though the parasite has not been found in South African wildlife and potential intermediate hosts may not be suitable (Boomker and Dale-Kuys, 1977). In the broader family Bovidae, *F. magna* infections have been investigated primarily in domestic cattle (Foreyt and Todd, 1976; Lee et al., 2016; Leontovyč et al., 2014). Both gross pathological and histopathological examination were consistent with previous reports of *F. magna* infection in aberrant hosts, with black coloration observed across the abdominal viscera (Foreyt and Todd, 1976) and both hepatic fibrosis and biliary hyperplasia observed under the microscope (Karamon et al., 2015; Mathieu et al., 2022; Marinković et al., 2013; Sommer et al., 2022). The absence of *F. magna* ova from feces and tissue collected from the suspected fluke capsule is consistent with the nature of dead-end host infections (Foreyt and Todd, 1976; Konjević et al., 2017).

Given the impala's apparent dead-end host status, these animals likely have little effect on the proliferation and ecology of *F. magna* on a larger scale. This finding is, however, of relevance to veterinary professionals and managers of game farms and zoological collections, particularly those in areas with established intermediate host populations and with concurrent American cervid and exotic bovid populations.

The PCR protocol described here, used to amplify fluke DNA found in host tissue, may help diagnosticians to circumvent difficulties associated with fluke infection confirmation. There are three common methods used to diagnose liver fluke infections, all of which face significant limitations in the diagnosis of dead-end host infections (Dowling et al., 2024). Total fluke counts (TFC) remain the "gold-standard" in fluke infection diagnostics but may be difficult to obtain in the field because of limited time or resources. Fecal egg counts (FECs) provide a post-necropsy diagnostic method but rely on the presence of identifiable ova in feces, as is uncharacteristic of dead-end host infections. FEC, as a liver fluke diagnostic method, is considered low sensitivity even in definitive host cases (French et al., 2016). There is a limited body of work detailing serological and coprological enzyme-linked immunosorbent assay (ELISA) development for *F. magna,* and with further validation, these tests may prove supreme as protocols and availability develops (Severin et al., 2015). These tests have certainly proven useful in the diagnosis of *Fasciola hepatica* infections (Brockwell et al., 2013; French et al., 2016; Sabatini et al., 2023). The approach detailed here may be especially useful for applications to dead-end host cases, where eggs are not shed in the feces and a liver tissue sample has been collected. The absence of fluke DNA from a host tissue sample does not eliminate the possibility of fluke infection, and the amplification of parasite DNA will depend on sampling location and quality. For example, it is suspected here that lung lesions were the result of ectopic fluke movement (as seen in cattle, Lee et al., 2016), but *F. magna* DNA was not isolated from a tissue sample. At the same time, the identification of fluke DNA in host tissue does not necessarily indicate an ongoing infection at the time of death. For these reasons, the methodology employed here is not appropriate for retrospective or passive surveillance of *Fascioloides magna* infection. Surveillance of *Fascioloides magna* in dead-end hosts using conventional methods is likely ineffective, and those hoping to prevent spillover in mixed species herds should instead focus on surveilling typical definitive hosts.

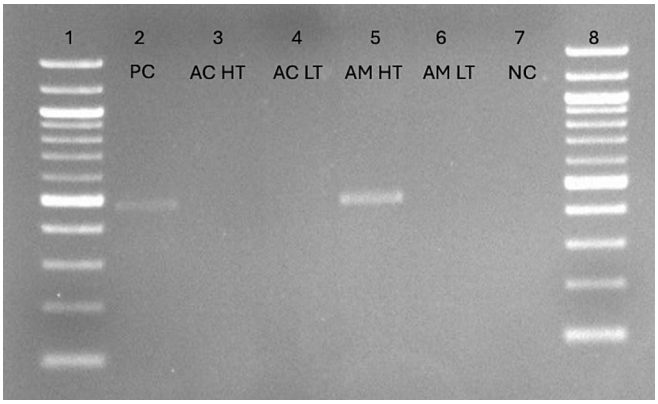

Fig. 2. Gel electrophoresis of cPCR product aimed at identifying trematode infection. "AC" refers to a blackbuck (*Antilope cervicapra*) tested in tandem with the impala ("AM"). "HT" refers to hepatic tissue, and "LT" to lung tissue.

5. Conclusion

In this case report, *F. magna* infection was detected in an impala using conventional PCR and confirmed by Sanger sequencing. Gross and histopathological lesions, as well as the absence of fluke ova in feces,

were indicative of a dead-end host infection. We emphasize the difficulties associated with identifying parasitic infections in dead-end hosts, potential approaches to surmounting those difficulties, and the value of describing potentially fatal dead-end host infections to veterinary professionals.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2025.101311.

CRediT authorship contribution statement

Braxton Sizemore: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **An-Chi Cheng:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Heather D.S. Walden:** Writing – review & editing, Resources. **Kuttichantran Subramaniam:** Writing – review & editing, Resources. **Samantha M. Wisely:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Juan M. Campos Krauer:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Ethics statement

Ethical approval was not required for the diagnostic research work performed in this study.

Funding

This research was funded by the University of Florida, Institute of Food and Agricultural Sciences, CHeRI, with funds provided by the Florida legislature #6000CHERI.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

We extend our gratitude to the Florida deer farms for providing the specimens, all UF CHeRI necropsy technicians for conducting fieldwork, the UF CVM Microbiology, Parasitology, and Serology Diagnostic Laboratory for bacterial pathogen identification, UF CVM Aquatic, Amphibian, and Reptile Pathology lab for histopathological examination, and UF CVM Diagnostic Parasitology laboratory for parasite examination.

Data availability

All data supporting the findings of this study are included in this article and its supplementary materials.

References

Boomker, J.D.F., Dale-Kuys, J.C., 1977. First Report of *Fascioloides magna* (Bassi, 1875) (in South Africa).

Brockwell, Y.M., Spithill, T.W., Anderson, G.R., Grillo, V., Sangster, N.C., 2013. Comparative kinetics of serological and coproantigen ELISA and faecal egg count in cattle experimentally infected with *Fasciola hepatica* and following treatment with triclabendazole. Vet. Parasitol. 196, 417–426. https://doi.org/10.1016/j.vetpar.2013.04.012.

Charlier, J., van der Voort, M., Kenyon, F., Skuce, P., Vercruysse, J., 2014. Chasing helminths and their economic impact on farmed ruminants. Trends Parasitol. 30, 361–367. https://doi.org/10.1016/j.pt.2014.04.009.

Corwin, R.M., 1997. Economics of gastrointestinal parasitism of cattle. In: Veterinary Parasitology, Fourth Ostertagia Workshop:Nematode Parasites of Importance to Ruminant Livestock, 72, pp. 451–460. https://doi.org/10.1016/S0304-4017(97)00110-6.

Cottingham, S.L., White, Z.S., Wisely, S.M., Campos-Krauer, J.M., 2021. A mortality-based description of EHDV and BTV prevalence in farmed White-tailed deer (*Odocoileus virginianus*) in Florida, USA. Viruses 13, 1443. https://doi.org/10.3390/v13081443.

Dowling, A., Lawrence, K.E., Howe, L., Scott, I., Pomroy, W.E., 2024. Assessment of accuracy of liver fluke diagnostic tests using the gold standard of total worm counts. Vet. Parasitol. Reg. Stud. Rep. 54, 101102. https://doi.org/10.1016/j.vprsr.2024.101102.

Foreyt, W.J., Todd, A.C., 1976. Development of the large American liver fluke, *Fascioloides magna*, in White-tailed deer, cattle, and sheep. J. Parasitol. 62, 26–32. https://doi.org/10.2307/3279036.

French, A.S., Zadoks, R.N., Skuce, P.J., Mitchell, G., Gordon-Gibbs, D.K., Craine, A., Shaw, D., Gibb, S.W., Taggart, M.A., 2016. Prevalence of liver fluke (*Fasciola hepatica*) in wild Red Deer (*Cervus elaphus*): coproantigen ELISA is a practicable alternative to faecal egg counting for surveillance in remote populations. PLoS ONE 11, e0162420. https://doi.org/10.1371/journal.pone.0162420.

Karamon, J., Larska, M., Jasik, A., Sell, B., 2015. First report of giant liver fluke (*Fascioloides magna*) in a farmed fallow deer (*Dama dama*) in Poland. J. Vet. Res. 59, 339–344. https://doi.org/10.1515/bvip-2015-0050.

Konjević, D., Bujanić, M., Erman, V., Gudan Kurilj, A., Živičnjak, T., Severin, K., Tomić, S., Martinković, F., 2017. New data on wild boar (*Sus scrofa* L.) a dead-end host for large American liver fluke (*Fascioloides magna*). Helminthologia 54, 77–80. https://doi.org/10.1515/helm-2017-0006.

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

Lee, J.K., Rosser, T.G., Cooley, J., 2016. Pulmonary embolization of immature *Fascioloides magna* causing fatal hemothorax confirmed by molecular technique in a heifer in the United States. J. Vet. Diagn. Invest. 28, 584–588. https://doi.org/10.1177/1040638716660129.

Leontovyč, R., Košťáková, M., Siegelová, V., Melounová, K., Pankrác, J., Vrbová, K., Horák, P., Kašný, M., 2014. Highland cattle and Radix labiata, the hosts of *Fascioloides magna*. BMC Vet. Res. 10, 41. https://doi.org/10.1186/1746-6148-10-41.

Lotfy, W.M., Brant, S.V., Ashmawy, K.I., Devkota, R., Mkoji, G.M., Loker, E.S., 2010. A molecular approach for identification of paramphistomes from Africa and Asia. Vet. Parasitol. 174, 234–240. https://doi.org/10.1016/j.vetpar.2010.08.027.

Malatji, M.P., Pfukenyi, D.M., Mukaratirwa, S., 2020. Fasciola species and their vertebrate and snail intermediate hosts in east and southern Africa: a review. J. Helminthol. 94, e63. https://doi.org/10.1017/S0022149X19000531.

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

Marinković, D., Kukolj, V., Aleksić-Kovačević, S., Jovanović, M., Knežević, M., 2013. The role of hepatic myofibroblasts in liver cirrhosis in fallow deer (*Dama dama*) naturally infected with giant liver fluke (*Fascioloides magna*). BMC Vet. Res. 9, 45. https://doi.org/10.1186/1746-6148-9-45.

Mas-Coma, S., Bargues, M.D., Valero, M.A., 2005. Fascioliasis and other plant-borne trematode zoonoses. Int. J. Parasitol. Paras. Zoonos. Emerg. Issues 35, 1255–1278. https://doi.org/10.1016/j.ijpara.2005.07.010.

Mathieu, A., Thacker, C., Teske, I., Jenkins, E., Wagner, B., Macbeth, B., Raverty, S., Pybus, M., 2022. *Fascioloides magna* in free-ranging rocky mountain bighorn sheep (*Ovis canadensis*). J. Wildl. Dis. 58, 592–598. https://doi.org/10.7589/JWD-D-21-00190.

Phiri, A.M., Mudenda, N.M., Luwe, M., Phiri, I.G.K., 2017. Use of bait containing triclabendazole against *Fasciola gigantica* in a herd of captive wild impala (*Aepyceros melampus*). J. Helminthol. 91, 376–379. https://doi.org/10.1017/S0022149X16000304.

Popovici, D.-C., Dărăbuș, G., Marin, A.-M., Ionescu, O., Moraru, M.M.F., Imre, M., Tîrziu, E., Mederle, N., 2024. Identification and molecular characterization of Giant liver fluke (*Fascioloides magna*) infection in European fallow deer (*Dama dama*) in Romania—first report. Microorganisms 12, 527. https://doi.org/10.3390/microorganisms12030527.

Sabatini, G.A., de Almeida Borges, F., Claerebout, E., Gianechini, L.S., Höglund, J., Kaplan, R.M., Lopes, W.D.Z., Mitchell, S., Rinaldi, L., von Samson-Himmelstjerna, G., Steffan, P., Woodgate, R., 2023. Practical guide to the diagnostics of ruminant gastrointestinal nematodes, liver fluke and lungworm infection: interpretation and usability of results. Parasit. Vectors 16, 58. https://doi.org/10.1186/s13071-023-05680-w.

Severin, K., Martinković, F., Janicki, Z., Marinculić, A., Slavica, A., Žele, D., Vengušt, G., Džaja, P., Vidić, Z., Konjević, D., 2015. Indirect ELISA and Western blotting as tools to diagnose fascioloidosis in a population of free-ranging red deer (*Cervus elaphus*). Veterinarski 85, 563–576.

Shanebeck, K.M., Besson, A.A., Lagrue, C., Green, S.J., 2022. The energetic costs of sub-lethal helminth parasites in mammals: a meta-analysis. Biol. Rev. 97, 1886–1907. https://doi.org/10.1111/brv.12867.

Sommer, M.F., Drdlicek, J., Müller, M., Thelemann, A., Just, F.T., 2022. *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*). Eur. J. Wildl. Res. 68, 73. https://doi.org/10.1007/s10344-022-01616-4.

Woolhouse, M.E.J., Taylor, L.H., Haydon, D.T., 2001. Population biology of multihost pathogens. Science 292, 1109–1112. https://doi.org/10.1126/science.1059026.