Detection of biliary trematodes in sheep and goats from northern and central Portugal

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Abstract

Fasciolosis and dicrocoeliosis are severe parasitic infections that result in substantial economic damages to livestock farming globally as a consequence of reduced productivity and viscera condemnation. Molecular tests such as polymerase chain reaction (PCR) can detect *Fasciola hepatica* and *Dicrocoelium dendriticum* DNA with high sensitivity and specificity. In this study, we aimed to assess the presence of *F. hepatica* and *D. dendriticum* by PCR-based techniques in 400 small ruminant bile samples retrieved from central Portugal. Additionally, we conducted genetic characterisation of *F. hepatica* and *D. dendriticum* in these samples. Only one of the 400 bile samples (0.25%; 95% confidence interval [CI]: 0.01–1.39) examined by PCR tested positive for *F. hepatica*, and two samples (0.50%, 95% CI: 0.06–1.79) tested positive for *D. dendriticum*. Our findings indicate a low prevalence of *F. hepatica* and *D. dendriticum* in Portuguese small ruminants, underscoring the need to investigate factors leading to meat rejection in slaughterhouses. Implementing effective parasite control measures is crucial to minimise economic losses and improve food safety. Addressing these infections and deploying targeted control strategies can enhance livestock production sustainability and ensure safe, high-quality meat products for consumers.

Key words: *Dicrocoelium dendriticum; Fasciola hepatica; Portugal; slaughterhouse; small ruminants*

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Introduction

Trematodes, commonly known as flukes, are flatworm parasites found in the liver, forestomach, or arteries of many animals, including humans. Some fluke species are particularly harmful in ruminants (Rojo-Vázquez et al., 2012). Sheep and other grazing animals are especially vulnerable to a range of fluke species (Arbabi et al., 2018), including the common liver fluke (*Fasciola hepatica*), the lancet fluke (*Dicrocoelium dendriticum*), and different rumen fluke species from the genera *Paramphistomum* and *Calicophoron* (Kahl et al., 2021).

Fasciolosis is a common foodborne zoonotic infection caused by *Fasciola hepatica* or *F. gigantica* that affects a wide variety of mammals, including humans (Utrera-Quintana et al., 2022). *Fasciola hepatica* is widespread across all continents, whereas *F. gigantica* is only found in Africa, the Middle East, and Asia (Caravedo et al., 2021). They undergo an intricate life cycle, utilising Lymnaeidae snails as an intermediate host, encysting as metacercariae on aquatic vegetation or other substrates, and requiring a definitive mammalian host, which can be cattle, sheep, or even humans. In the human population, this parasitic infection is recognised as a re-emerging disease in a number of countries, and its spread is closely linked to the prevailing climatic conditions (Mas-Coma et al., 2005). Nearly 80 species of intestinal flukes infest both humans and animals globally (Mas-Coma et al., 2009; Fürst et al., 2012). Nevertheless, in South America, only *F. hepatica* has been confirmed in both humans and cattle (Mas-Coma et al., 2009). Fasciolosis is acknowledged as a significant endemic illness in this region of the American continent (Mas-Coma et al., 2005; Carmona and Tort, 2017; Molento et al., 2018; Américo et al., 2022).

Bovine fasciolosis is present on all continents except Antarctica, posing a potential risk to over 700 million animals. The global economic impact of *F. hepatica* infection in cattle is projected to exceed USD 3 billion annually, affecting the farming and industry sectors (Hayward et al., 2021). The financial impact of this expense is not extensively measured at national or regional scales, and there are reports indicating that fluke negatively influences milk production and the composition of carcasses, thereby extending the time needed to achieve suitable weight for slaughter (Howell et al., 2015; Mehmood et al., 2017; Kouam et al., 2019). Therefore, it is important to continue improving techniques for the detection of liver fluke infections.

Dicrocoeliosis is a zoonosis caused by *Dicrocoelium dendriticum*, *D. hospes*, *D. chinensis*, and *D. suppereri*. The geographic distribution of these species varies, with *D. dendriticum* present in Europe, Asia, Northern Africa, and North America (Rojo-Vázquez et al., 2012). A third species, initially extracted from musk deer (*Moschus moschiferus*) in the Baikal region of the former Soviet Union, has been identified as *D. orientalis* (Hinaidy, 1983) and was later renamed *D. chinensis* (Otranto et al., 2007) due to the prior assignment of the initial name and its morphological resemblance to *D. chinensis* identified in sheep from China (Hinaidy, 1983; Schuster and Ramirez-Avila, 2008). It has been documented in various cervid species within the former Soviet Union (Otranto et al., 2007) and sika deer (*Cervus nippon centralis*) in Japan (Taira et al., 2006).

The transmission of dicrocoeliosis occurs in lowland or mountain pastures that offer suitable conditions for the survival and growth of snails and ant species serving as intermediate hosts (Otranto and Traversa, 2003). In Europe, *D. chinensis*

has been detected in mouflon (*Ovis ammon musimon*) and roe deer (*Capreolus capreolus*) in Austria (Hinaidy, 1983), as well as in mouflon in northern Italy (Poglayen et al., 1996). Unlike our comprehensive understanding of the ecology, biology, and pathogenicity of *D. dendriticum*, information pertaining to *D. chinensis* is limited and only accessible for China (Otranto et al., 2007). However, distinguishing between *D. chinensis* and *D. dendriticum* is easily achieved based on the size and orientation of the testes (Hinaidy, 1983; Otranto et al., 2007). Research involving molecular and biochemical examinations of the population structure of *Dicrocoelium* spp., both within individual hosts and across different hosts, is currently limited to *D. dendriticum* (Campo et al., 1998; Sandoval et al., 1999). For instance, an analysis of isoenzymes in *D. dendriticum* collected from cattle, sheep, and goats indicated minimal phenotypic variation among flukes originating from a single host individual or from a single host species in the same geographical area (Campo et al., 1998). Nevertheless, an examination of genetic characteristics using randomly amplified polymorphic DNA uncovered significant variability within the population among specimens gathered from sheep within a relatively compact geographical region (Sandoval et al., 1999).

Ribosomal DNA (rDNA) has been extensively employed in phylogenetic and diagnostic investigations of parasitic nematodes (Blouin, 2002; Gasser and Newton, 2000) and flatworms (Olson and Tkach, 2005). However, there have been limited molecular investigations on the order Plagiorchiida (Tkach et al., 2000, 2001). Despite dedicated endeavours to categorise these trematode species, namely *Fasciola* spp. and *Dicrocoelium* spp., by relying on morphological traits like body length and width, the task of precise discrimination persists as a challenge. This complexity arises from a multitude of influencing factors, including the age of flukes, the diverse range of host species involved, and the intricate technical challenges encountered in the fixation process (Kendall, 1965).

Fascioliosis and dicrocoeliosis are considered major parasitic diseases that cause substantial economic losses to livestock farming around the world due to decreased production and viscera condemnation (Arbabi et al., 2018). In both industrialised and developing countries, an estimated 10-80% of dairy and meat herds are infected, costing the sector a total of USD 3 billion in 1994 (Caravedo and Cabada, 2020). Reduced milk and wool production, lower carcass weight and composition, lower fertility and costs with treatment are other impacts caused by infections with these parasites (Caravedo et al., 2021).

Currently, the most commonly employed technique for examining faecal samples to detect *F. hepatica* eggs is the traditional sedimentation method. This approach is both straightforward and cost-effective, requiring only fundamental laboratory equipment (Boray, 1969). A newly introduced approach for diagnosing *F. hepatica* involves the "FLUKEFINDER®" method (FLUKEFINDER® Diagnostic System, Soda Springs, Idaho, USA). This method relies on a combination of selective sieving followed by a sedimentation process (Zárate-Rendón et al., 2019; Reigate et al., 2021). Furthermore, other wet mount and/or condensation techniques are also used, such as formalin-ether and Telman or Kato-Katz tests, which are frequently used to detect parasite eggs in faeces, bile, or fluid in the duodenum. Also, certain serological methods like Fas2-enzyme-linked immunosorbent assay (Fas2-ELISA), immunofluorescence

assay, and indirect hemagglutination assay can be used for the diagnosis of the disease at any point during its progression (Amiri et al., 2021). In endemic countries, the primary diagnostic method typically employed is stool microscopy (Caravedo and Cabada, 2020), although the most effective concentration techniques rely on devices that come with drawbacks such as high cost, difficulty in implementation for extensive surveys, and limited accessibility (Caravedo and Cabada, 2020).

Coproscopical, immunological, and biochemical methods are used to diagnose dicrocoeliosis in live animals (Campo et al., 2000; González-Lanza et al., 2000; Manga-González et al., 2004; Ferreras-Estrada et al., 2007; Sandoval et al., 2013). Coproscopical methods involve examining faecal samples for the presence of *Dicrocoelium* eggs or other diagnostic stages using techniques such as flotation or sedimentation (Sandoval et al., 2013). Immunohistochemical studies (Ferreras-Estrada et al., 2007), can provide insights into the immune response of the host to *Dicrocoelium* infection. Additionally, IgG antibody responses to excretory-secretory or somatic antigens of *Dicrocoelium dendriticum* in experimentally infected sheep can be indicative of infection (González-Lanza et al., 2000). Biochemical methods involve assessing hepatic marker enzymes, biochemical parameters, and pathological changes in the liver associated with *Dicrocoelium* infection (Manga-González et al., 2004).

While *F. hepatica* primarily infects the liver and causes hepatic lesions, *Dicrocoelium* primarily affects the bile ducts (Otranto and Traversa, 2003). Furthermore, the diagnostic methods for *Dicrocoelium* often focus on coproscopical and immunological approaches (Ferreras-Estrada et al., 2007; Sandoval et al., 2013), whereas *F. hepatica* diagnosis may also involve sedimentation techniques and other wet mount and/or condensation techniques (Boray, 1969; Amiri et al., 2021). Additionally, the pathological effects and immune responses elicited by these two parasites may differ, as evidenced by the varied experimental studies cited (Manga-González et al., 2004).

Despite the above, detection of *F. hepatica* and *D. dendriticum* DNA using molecular tests such as polymerase chain reaction (PCR) has been recognised as having higher sensitivity and specificity (Caravedo and Cabada, 2020).

The aim of this study was to assess the presence of *F. hepatica* and *D. dendriticum* by PCR-based techniques in the bile of small ruminants of Portugal. Moreover, genetic characterisation of *F. hepatica* and *D. dendriticum* was performed for the first time in the country.

Materials and methods

Geographical aspects

The animals included in this study originated from their respective native habitats. The geographical focus of this research is the Estrela Mountain range (Serra da Estrela) situated in central Portugal. In this region, the National Association of Serra da Estrela Sheep Breeders (http://www.ancose.com) oversees the management of the indigenous Portuguese sheep breed "Serra da Estrela". The study area is characterised as a semi-natural Mediterranean pasture, typified by its rugged, mountainous terrain interspersed with shrubby and herbaceous layers, which are commonly utilised for sheep grazing (Monteiro et al., 2020).

There are only 215 registered Serra da Estrela sheep flocks with a total registered number of 18,603 animals. There is minimal animal movement outside the farm

boundaries, reflecting the breed's localised and controlled production practices and no animals can be introduced to the area which consequently would imply the loss of the protection of geographical indications and designations of origin for agricultural products and foodstuffs (Monteiro et al., 2020).

Sample collection

A total of 400 bile samples were obtained from individual sheep (*n*=335) and goats (*n*=65), at a small ruminant slaughterhouse located in Serra da Estrela subregion in central Portugal. The slaughterhouse operates five days a week and slaughters approximately 42,000 animals every year, mainly juveniles. Pre- and post-mortem inspections are performed on all animals by an official veterinarian. Samples were collected between January and March 2022 by a trained field worker. Of the 400 animals screened for biliary trematodes, 51 were adult sheep (12+ months), 284 lambs (3–12 months), 18 adult goats (12+ months), and 47 kids (3–12 months). All sampled animals were from the northern and central regions of Portugal. Bile samples (5 mL) were individually collected directly from the biliary bladder in the slaughter line (Caravedo et al., 2021). No animals were killed for the sake of this study. All bile samples were kept at 4°C and transported to the laboratory within 12 hours. Samples were then stored at -20°C until DNA extraction, which was completed within two weeks of collection.

DNA extraction

DNA was extracted from 200 µL of each individual bile sample using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, in the QIAcube® automated platform (Qiagen, Hilden, Germany). Eluted DNA was stored at -80°C with DNase/RNase-free water until further analysis.

Molecular detection of Fasciola spp. and Dicrocoelium spp.

To detect *Fasciola* spp. in bile samples, a conventional PCR assay was used to amplify a 493 bp fragment of the mitochondrial cytochrome c oxidase I (COI) gene with the primer set Ita8-Forward/Ita9-Reverse, according to Itagaki et al. (2005). To detect *Dicrocoelium* spp., a conventional PCR assay was used to amplify a 600 bp fragment of the ribosomal internal transcribed spacer 2 (ITS2) plus 5.8S and 28S flanking regions with the primer set Dd58SF1/Dd28SR1, accordingly to Otranto et al. (2007).

Oligonucleotides used for the molecular detection of *Fasciola* spp. and *Dicrocoelium* spp. described above are shown in Table 1. All end-point PCR reactions were run on a T100 thermocycler (Bio-Rad). The Xpert Fast Hotstart Mastermix 2x with dye (GRiSP®, Porto, Portugal) was used and the reaction mixtures were prepared according to the manufacturer's instructions. Thermocycling conditions were as follows: initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 56°C (*Fasciola* spp.)/53°C (*Dicrocoelium* spp.) for 15 seconds, and extension at 72°C for 2 seconds and a final extension at 72°C for 10 minutes. The amplified DNA fragments were detected by subjecting the PCR amplification products to electrophoresis on 1% agarose gels stained with Xpert Green Safe DNA gel dye (GRiSP®, Porto, Portugal) at a voltage of 120 V for 30 minutes. UV light was used to validate and verify the obtained outcomes.

Sequencing and phylogenetic analysis

The GRS PCR & Gel Band Purification Kit (GRiSP®, Porto, Portugal) was used to purify amplicons that exhibited a positive

Table 1. Oligonucleotides used for the molecular identification and/or characterisation of *Fasciola* spp. and *Dicrocoelium* spp.

Target organism	Locus	Oligonucleotide	Sequence (5´–3´)	Reference
Fasciola spp.	COI	Ita8-Forward	ACGTTGGATCATAAGCGTGT	(Itagaki et al., 2005)
		Ita9-Reverse	CCTCATCCAACATAACCTCT	
Dicrocoelium spp.	ITS-2	Dd58SF1	ATATTGCGGCCATGGGTTAG	(Otranto et al., 2007)
		Dd28SR1	ACAAACAACCCGACTCCAAG	

signal and were of the expected size. Following purification, the Sanger method was employed along with specific primers designed for the targeted genes (Itagaki et al., 2005; Otranto et al., 2007). Bidirectional sequencing was performed, and the obtained sequences were aligned and compared to sequences from the NCBI (GenBank) nucleotide database, accessed on 3 November 2022 (http://blast.ncbi.nlm.nih.gov/Blast), and the alignment and comparison were carried out using the BioEdit Sequence Alignment Editor v7.1.9 software, version 2.1. For phylogenetic analysis, the software MEGA version X (Kumar et al., 2018) and the Interactive Tree Of Life (iTOL) platform were applied. The analysis incorporated sequences obtained in this study, as well as representative sequences obtained from GenBank. The maximum-likelihood (ML) approach was used to conduct the analysis (Tamura, 1992; Kumar et al., 2018). To estimate the ML bootstrap values, the Hasegawa-Kishino-Yano model (*Fasciola* spp.) and the General Time Reversible model (*Dicrocoelium* spp.) were employed with 1000 replicates (Tamura, 1992). MEGA version X (Kumar et al., 2018) determined this model to be the most suitable replacement model. The sequences obtained in this study were deposited in GenBank with accession numbers OQ980481 (*F. hepatica*), OR060629 and OR074512 (*D. dendriticum*).

Statistical analysis

The occurrences of both trematode species in small ruminants from a slaughterhouse in Portugal were calculated based on the proportion of positive samples to the total number of samples examined with a 95% confidence interval (95% CI).

Results

Among the total 400 bile samples assessed by PCR, one was found to be positive for *Fasciola* spp. (0.25%; 95% confidence interval [CI]: 0.01–1.39) and two were positive for *Dicrocoelium* spp. (0.50%, 95% CI: 0.06–1.79). *Fasciola* spp. was detected in an adult female sheep (Figure 1) and *Dicrocoelium* spp. in two adult female sheep (Figure 2).

Bidirectional Sanger sequencing of the positive samples was performed, followed by nucleotide BLAST analysis. Phylogenetic analysis further categorised the positive samples as *F. hepatica* or *D. dendriticum*, as shown in Figures 3 and 4. Sequence analyses of the obtained amplicons showed the positive *F. hepatica* sample to be 100% identical to a *F. hepatica* COI sequence retrieved from a donkey in Iran (MF537586). Both *D. dendriticum* positive samples showed high similarities to a *D. dendriticum* ITS-2 sequence from a ruminant in Iran (MN831475): one showed to be 99.81% and the other one 99.82% identical.

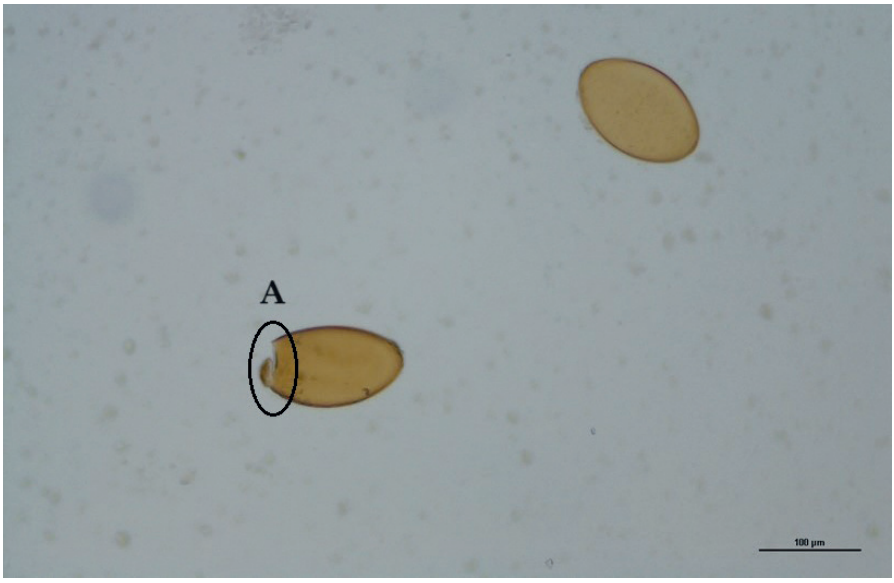

Figure 1. Microscopic visualisation of *Fasciola hepatica* eggs from the positive sample (scale bar = 100 µm). Eggs of *Fasciola* spp. are broadly ellipsoidal, operculated, 130-150 µm long by 60-90 µm wide. The circle shows an operculum that has popped open at its target breaking point (A).

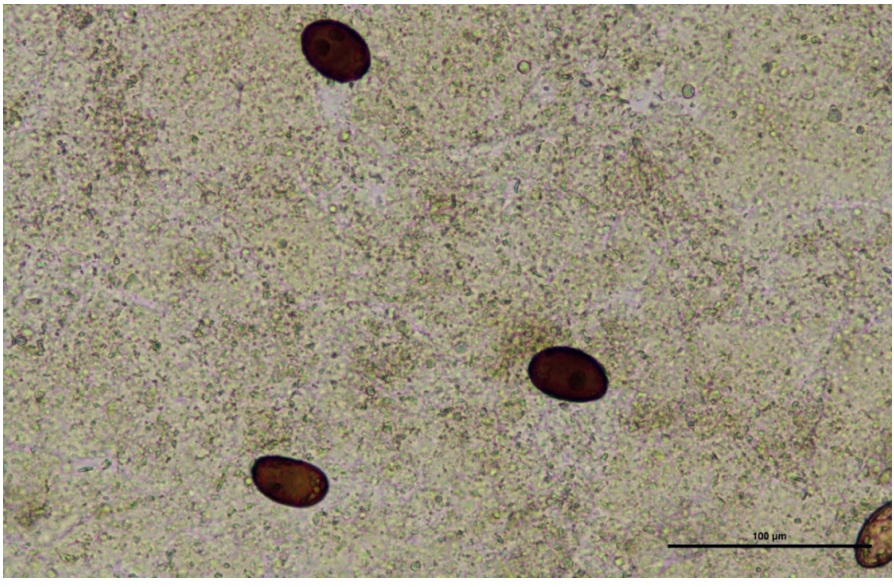

Figure 2. Microscopic visualisation of *Dicrocoelium dendriticum* eggs from a positive sample (scale bar = 100 µm). Eggs of *Dicrocoelium dendriticum* are operculated, 35–45 µm long by 20–30 µm wide. The eggs are thick-shelled and usually dark brown.

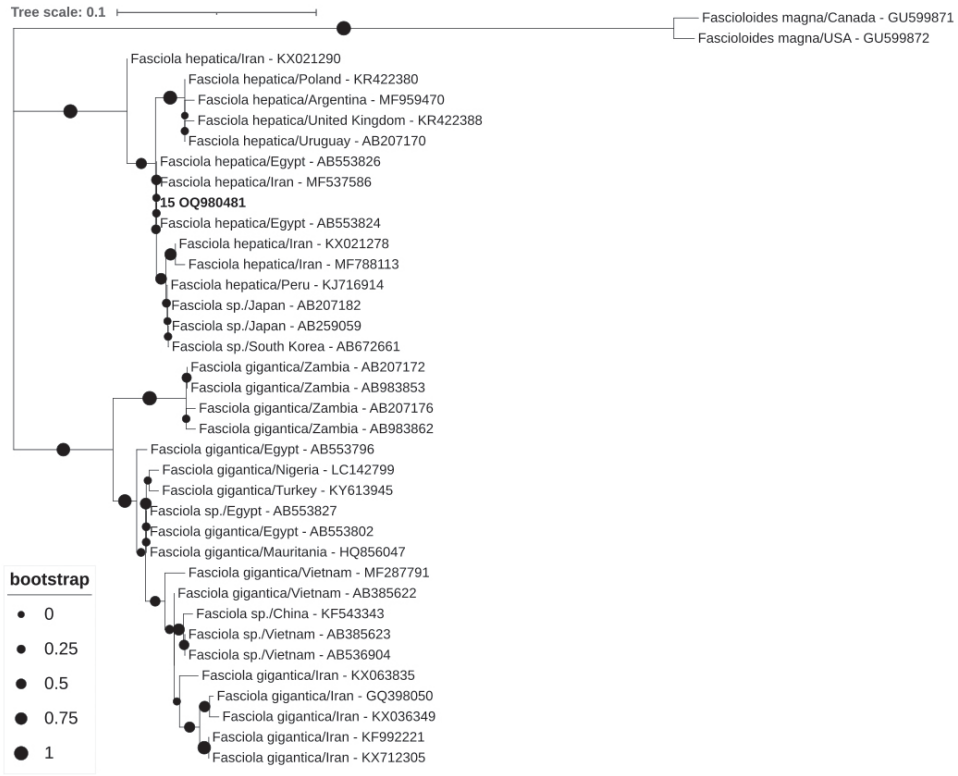

Figure 3. Phylogenetic analysis of *Fasciola hepatica* found in small ruminants. Tree inferred using the MEGA X maximum likelihood method (Hasegawa-Kishino-Yano model) and the Interactive Tree of Life (iTOL) based on 37 nucleotide *Fasciola* sequences at the COI marker, including the sequence found in this study (*F. hepatica*, plus its accession number, is in bold) and 19 strains of the two *Fasciola* spp. obtained from GenBank (the identification of each item was performed using an accession number, along with the species and country of origin, without the use of bold or shading).

Discussion

Infections by *F. hepatica* and *D. dendriticum* affect the liver and bile ducts of a range of animal species, including cattle, goats, and sheep, and are the leading source of liver rejection in slaughterhouses, causing impactful economic losses to livestock farming worldwide (Aminzare et al., 2018; Borji and Parandeh, 2010; Mohamed, 2021; Theodoropoulos et al., 2002). They are also responsible for economic losses because of weight loss, decreased milk production, decreased fertility, growth retardation, and costs associated with therapy in the treatment of frequent secondary bacterial infections, and in some cases the animal's death (Sanchez-Vazquez and Lewis, 2013; Mazeri et al., 2017).

Visual liver inspection is an important public health measure that has been put into place to control transmission to humans; nevertheless, it lacks sensitivity, which is one of the drawbacks of this meth-

Figure 4. Phylogenetic analysis of *Dicrocoelium* spp. found in small ruminants. Tree inferred using the MEGA X maximum likelihood method (General Time Reversible model) and the Interactive Tree of Life (iTOL) based on 26 nucleotide sequences at the COI marker, including the sequence found in this study (*Dicrocoelium dendriticum*, plus its accession number, is in bold) and 24 strains of different trematode species obtained from GenBank (identification of each item based on an accession number, along with the species and country of origin, without the use of bold or shading).

od (Rapsch et al., 2006; Takeuchi-Storm et al., 2017). Another limitation is that statistics collected from slaughterhouses are not very reliable, with the possibility of animals with a low parasite burden going undetected (Utrera-Quintana et al., 2022).

Even though slaughterhouse surveys have certain drawbacks, they are a cost-effective method of acquiring data about cattle and small ruminant diseases albeit relying mostly on visible abnormalities that could compromise meat hygiene and safety (Mohamed, 2021).

Of the 400 bile samples assessed by PCR in this study, one tested positive for *F. hepatica*, and two for *D. dendriticum*. All three positive bile samples originated from adult sheep.

Considering the small number of cases detected (one *F. hepatica* and two *D. dendriticum*), it is not possible to suggest a higher susceptibility or vulnerability of sheep to one parasite over the other. Moreover, given the notably low infection rate reported here, there appears to be a lesser urgency in emphasising the necessity for control programs, treatment, and zoonosis prevention in the area.

In this study, PCR confirmed that the liver parasitic burden is very low in the region and that sanitary approval of this viscera could be considered. It should be taken into account that most of the sampled animals were young (< 1 year old), a circumstance which could have somehow reduced the likelihood for parasitic

exposure and infection. However, certain PCR assays have the capability to identify trematode DNA several weeks prior to the detection of eggs in faecal samples (Caravedo et al., 2021).

When comparing the occurrence of infection with *F. hepatica* and *D. dendriticum* found in this study (0.25% and 0.50%, respectively), it aligns with other reports from Portugal, where *D. dendriticum* occurrence in sheep was higher than that of *F. hepatica*. A study involving indigenous sheep breeds in Portugal reported a 22.5% occurrence of *Dicrocoelium* spp. compared to a 1.8% occurrence for *F. hepatica* out of 512 animals (Ruano et al., 2019). However, caution is needed when comparing occurrence data from different studies, as different detection methods employed in each study may vary in their diagnostic sensitivities. Also, it is important to note that a considerable number of the sampled animals were of young age, which in this situation might have decreased the chances of exposure to or infection by these parasites. In another study where *F. hepatica* IgG antibodies were assessed in a population of confined sheep from central Portugal, a low circulation of *F. hepatica* was found (19.6% and 18.5% in the first and second years of the study, respectively) (Coelho et al., 2021). Moreover, although there were no discernible differences between sheep and goats in terms of worm burden and faecal egg count, sheep appear to be more vulnerable to *D. dendriticum* (Otranto and Traversa, 2002).

In a survey in nearby northwestern Spain, a sheep flock was tested for *F. hepatica* with a standard coproscopical sedimentation method and indirect enzyme-linked immunosorbent assay (ELISA). The occurrence of *F. hepatica* infection found by ELISA was 77.6%, whereas the occurrence by the coproscopical sedimentation method was 23.7%. However, antibodies persist even if the infection has cleared, while coproscopical methods can only detect current infections. Moreover, mixed infections of *F. hepatica* and *D. dendriticum* were also detected, with occurrences of 11.2% and 35.5% by coproscopical sedimentation and ELISA, respectively (Ferre et al., 1995). In a seroepidemiological study in southern Spain for detection of antibodies to liver trematodes in sheep, goats, and deer, *F. hepatica* antibodies were detected only in deer, with a 3% occurrence, while *D. dendriticum* antibodies were detected in 1% of sheep/goats and 4% of deer (Arias et al., 2012).

Currently, only limited data are available on the occurrence of trematode infections in small ruminants such as sheep and goat in Portugal, as most surveys are made only on cattle (Arias et al., 2011; Barbosa et al., 2019; Conceição et al., 2004). When it comes to *F. hepatica* and *D. dendriticum* infections, most studies are focused on the former, with a gap in knowledge regarding *D. dendriticum* infection (Arias et al., 2012). Nonetheless, the present study underscores the importance of meat inspection records in the context of liver parasitic infections in Portugal, particularly for *F. hepatica* and *D. dendriticum*, which are medically significant human parasites. This highlights the need for control and prevention strategies for these trematodes. Deworming sheep for *Fasciola* spp. and *Dicrocoelium* spp. is recommended due to their zoonotic nature. It is important to effectively communicate the impacts of these zoonoses and the value of routine parasitological testing to sheep producers and veterinarians.

Conclusions

Our findings indicate that *F. hepatica* and *D. dendriticum* circulate at considerably low levels among small ruminants in northern and central Portugal. This

suggests that current control measures may be effective, contributing to low infection rates. However, it remains important to continue monitoring and understanding the factors contributing to meat rejection in slaughterhouses. Maintaining and potentially improving parasite control techniques can help ensure these low levels are sustained, thereby minimising economic losses and enhancing food safety standards. By continuously addressing these parasitic infections and implementing effective control measures, we can support the economic sustainability of livestock production and ensure safe and high-quality meat products for consumers.

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Otkrivanje jetrenih metilja u ovaca i koza iz sjevernog i središnjeg Portugala

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Fascioloza i dikrocelioza ozbiljne su parazitarne infekcije koje prouzroče znatne ekonomske štete stočarstvu diljem svijeta kao posljedica smanjene produktivnosti i uništavanja unutarnjih organa. Molekularni testovi poput lančane reakcije polimerazom (PCR) mogu otkriti DNK *Fasciola hepatica* i *Dicrocoelium dendriticum* parazita s visokom osjetljivošću i preciznošću. U ovoj studiji cilj je bio procijeniti prisutnost parazita *F. hepatica* i *D. dendriticum* pomoću tehnika na bazi PCR-a u 400 uzoraka žuči malih preživača prikupljenih iz središnjeg Portugala. Uz to, provedena je genetska karakterizacija parazita *F. hepatica* i *D. dendriticum* u tim uzorcima. Samo jedan od 400 ispitanih uzoraka žuči (0,25 %; 95 % interval pouzdanosti [CI]: 0,01–1,39) ispitanih PCR-om bili su pozitivni na *F. hepatica*, a dva uzorka (0,50 %, 95 % CI: 0,06–1,79) bila su pozitivna na *D. dendriticum*. Naši nalazi ukazuju na nisku raširenost *F. hepatica* i *D. dendriticum* u portugalskih malih preživača, naglašavajući potrebu za istraživanjem čimbenika koji dovode do odbijanja mesa u klaonicama. Provedba učinkovitih mjera kontrole parazita ključna je za smanjenje ekonomskih gubitaka i poboljšanje sigurnosti hrane. Rješavanje ovih infekcija i uvođenje ciljanih strategija kontrole može povećati održivost proizvodnje životinja i osigurati sigurne, visokokvalitetne mesne proizvode za potrošače.

Ključne riječi: *Dicrocoelium dendriticum, Fasciola hepatica, Portugal, klaonica, mali preživači*