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Fasciolosis in the Mediterranean island of Corsica (France): Insights from epidemiological and malacological investigations

A. Alba [a,b,*], S. Grech-Angelini [c], A.A. Vázquez [b,d], P. Alda [d,e], Q. Blin [a], L. Lemmonier [a], A. Chauvin [f], C. Chartier [f], P. Douchet [g], S. Hurtrez-Boussès [d,h,i], O. Rey [g], J. Foata [a], J. Boissier [g], Y. Quilichini [a]

[a] Laboratoire de Sciences Pour l'Environnement, UMR 6134, CNRS, Université de Corse Pasquale Paoli, Corte, Corse, France
[b] Centro de Investigaciones, Diagnóstico y Referencia, Instituto de Medicina Tropical "Pedro Kourí", La Habana, Cuba
[c] Groupement Technique Vétérinaire de Corse, Corse, France
[d] MIVEGEC, Université de Montpellier, CNRS, IRD, Montpellier, France
[e] Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS–CCT–CONICET Bahía Blanca), Bahía Blanca, Argentina
[f] UMR 1300 BIOEPAR Oniris, Nantes, France
[g] IHPE, Univ. Montpellier, CNRS, Ifremer, Univ. Perpignan Via Domitia, Perpignan, France
[h] CREES, Montpellier, France
[i] Département de Biologie-Écologie, Faculté des Sciences, Université de Montpellier, Montpellier, France

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ABSTRACT

Fasciolosis is a re-emergent parasitic disease of worldwide significance with a major global impact on livestock health and production. In the French Mediterranean island of Corsica, fasciolosis has been recognized for a long time but little is known about its dynamic as the main investigations are outdated. Three compartments - definitive domestic hosts, intermediate hosts and environment - involved in fasciolosis transmission were studied by applying an integrative and extensive approach: (1) farm and abattoir surveys, (2) snail sampling, identification and infection prospection, and (3) snail habitat analysis; and (4) a questionnaire-based survey to inquire about husbandry practices and environmental risks. Our results indicate a significant circulation of the liver flukes in Corsican livestock, with 90% (252/279) of the sampled farms testing positive for anti-*F. hepatica* antibodies. At the abattoir, 46% (67/149) of cattle were positive for *F. hepatica* antibodies and eggs were present in the bile of 19% (26/139) bovines. In addition, high prevalence of *Dicrocoelium dendriticum* (69%) was observed in slaughtered cattle. Malacological surveys registered the occurrence of several lymnaeid species in a variety of habitats throughout the island. In particular, we report for the first time the presence of the invasive lymnaeid snail *Pseudosuccinea columella* in Corsica, a potential intermediate host for *F. hepatica*. We also found that the presence of *Galba truncatula* and, to a lesser extent, that of *Peregriana peregra,* is associated with altitude. *Fasciola hepatica* DNA was detected in the latter species occurring at two different sites. Finally, a questionnaire-based study revealed risky management practices among Corsican farmers, low perception of transmission and a suboptimal use of flukicide treatments as main control strategy. Our results show that animal fasciolosis in

* Corresponding author at: Laboratoire de Sciences Pour l'Environnement, UMR 6134, CNRS, Université de Corse Pasquale Paoli, Corte, Corse, France.
E-mail address: annia.alba@gmail.com (A. Alba).

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Corsica is characterised by a significant circulation and a favourable epidemiological scenario for transmission to occur.

1. Introduction

Among the re-emergent human parasitic infections, fasciolosis stands out as one of the most important snail-borne diseases due to its significant impact on human and livestock health and to livestock production worldwide (Mehmood et al., 2017; Nyindo and Lukambagire, 2015). The successful spreading of fasciolosis observed nowadays is mostly associated with the species *Fasciola hepatica*, a food/water-borne digenean trematode presenting a dioxenic life cycle that includes the development of free-living stages, i.e. egg, miracidium and metacercaria, with the alternation of intra-host parasitic stages that develop inside a lymnaeid snail (intermediate host) and a mammal (e.g. human, ruminant livestock or wildlife; definitive host).

Being a zoonotic parasite with a high adaptive capacity to an uncommon range of intermediate and definitive hosts (Cwiklinsky et al., 2015), and with a close association with economic activities (mainly cattle husbandry; Sabourin et al. (2018)), *F. hepatica* also exhibits one of the widest distributions within vector-borne pathogens. It occurs from tropical to temperate regions in all inhabited continents, and presents a remarkable altitudinal pattern of transmission (Parkinson et al., 2007; Roldán et al., 2020). In Europe, *F. hepatica* is known to be a significant veterinary problem with several regions characterised by a marked endemicity in countries such as France, Spain or the United Kingdom (Martínez-Valladares et al., 2013; Pritchard et al., 2005; Vignoles et al., 2019). Spatial modelling analyses of past and future climatic suitability for *F. hepatica* transmission in Europe from a climate change perspective supported its significant increase from the late 1990s to early 2000s, while forecasting an even higher increase, mainly in central and north-western Europe and some regions of the Mediterranean basin, including the French island of Corsica (Caminade et al., 2015).

Corsica, the fourth Mediterranean island in size (180 km long and 70 km wide), is located in proximity to metropolitan France and the Italian peninsula, and 15 km north of the island of Sardinia (Italy). It is the wettest, most forested (46% of the territory covered by green forests, woodlands and shrubs) and the most mountainous of all Mediterranean islands. A wide mountain range bisecting the island covers two thirds of its territory, with Monte Cintu peaking at 2706 m and several other summits over 2000 m. This particular topography determines different microclimates depending on the altitude and sunlight exposure, with a typical Mediterranean climate of warm dry summers and mild rainy winters along the coast, but more markedly temperate weather observed at increasing altitudes, and a typical alpine climate pattern above 1500 m (Mouillot et al., 2008). The island is a sparsely populated region (about 330,000 inhabitants) where extensive livestock farming is an important economic activity.

As is the case in Continental Europe, *F. hepatica* is the only *Fasciola* species so far described in Corsica, and livestock (mainly cattle and sheep) are the main definitive hosts (Gretillat, 1963). Importantly however, small wild mammals, particularly the black rat *Rattus rattus,* are recognized as other significant definitive hosts on the island (Magnanou and Morand, 2006). Conversely, reports of human infection are rather infrequent with only 42 cases documented between 1952 and 1990 (WHO, 1995). Five species of lymnaeid snails that could potentially host *Fasciola* spp. (Vázquez et al., 2018) have been acknowledged in Corsica: *Galba truncatula*, *Peregriana peregra* (syn. *Peregriana labiata* according to Vinarski et al. (2020)), *Ampullaceana auricularia*, *A. balthica*, and *Stagnicola fuscus* (Glöer, 2019). Little is known, however, about current dynamics of fasciolosis in Corsica as no major investigations focused on this topic have been carried out over the last decades. The growth of the Corsican livestock industry and the already noticeable temperature increase associated with global warming in the island (Serpentini et al., 2017) make even more urgent the need for updated and more comprehensive studies aimed at exploring the epidemiological scenario of this parasitosis in Corsica.

In the present paper, we describe the latest and most complete eco-epidemiological views on fasciolosis in Corsica using a diverse set of approaches that explore the different compartments related to the transmission of *F. hepatica:* the domestic definitive host, the intermediate host, and the environment (husbandry practices). Classical epidemiological (data revision, abattoir survey, questionnaire-based survey) and malacological (ecology and infection status of lymnaeid snails) investigations, with the support of immunoenzymatic and molecular techniques, were carried out. Our results clearly indicate that fasciolosis presents a complex epidemiological scenario in Corsica that has been largely overlooked. These results highlight the necessity of a drawing attention to this parasitic disease.

2. Material and methods

2.1. The definitive host: Epidemiological screenings of F. hepatica in livestock

2.1.1. Farm survey: Serological screening of anti-F. hepatica antibodies in sheep and cattle from Corsica

We have included a previously performed and unpublished farm survey carried out as part of the national surveillance monitoring of animal diseases. Veterinarians collected cattle and sheep blood samples from January to June 2018 from 1690 adult cattle (>2 years-old) and 1835 adult sheep (>1 year-old) sampled from 279 farms (155 bovine and 124 ovine farms). These represent 15% and 23% of the cattle and sheep farms of the island, respectively. The collected animals originated from 49% (149/298) of the Corsican municipalities with ruminant farming activities (Grech-Angelini et al., 2020). On average, 10 blood samples per cattle farm (ranging from 9 to 13) and 15 per sheep farm (ranging from 12 to 17) were included in this study.

Bovine blood samples were tested by a commercial ELISA kit for specific antibodies detection based on the excretion-secretion proteins of *F. hepatica* (SVANOVIR® *F. hepatica*-Ab, Svanova Biotech, Uppsala). According to the manufacturer's instructions, all

ELISA results were expressed as optical density ratios (ODR): ODR = (OD_{sample} – $OD_{negative\ control}$)/($OD_{positive\ control}$ – $OD_{negative\ control}$). Sheep samples were analysed by an in-house ELISA using 96-well plates sensitized with total excretion-secretion antigen of *F. hepatica* and laboratory standardized negative and positive controls (Chauvin et al., 1995; Chauvin et al., 2001). The ODRs were calculated for both ELISAs and a cut-off value for positive sample discrimination of ODR > 0.4 was used.

Two different evaluation approaches were applied for sample testing. The first approach consisted in testing 705 batches of sera (1 batch = 5 pooled sera consisting of 1 μL of each individual serum] collected within the same farm in 495 μL of sampling buffer), to estimate the positivity of the sampled farms to *F. hepatica*. Whenever possible, two batches per bovine farm and three batches per ovine farm were used as biological replicates. A farm was considered positive if at least one of the serum batches resulted positive (ODR > 0.4). The second approach used 380 sera (230 from sheep and 150 from cattle) randomly selected among positive batches (ODR ≥ 0.4) to be individually tested (5 μL of serum to 495 μL of sampling buffer). Seroprevalence per species within randomly selected positive farms was estimated as the percentage of positive individually-tested animals and confidence intervals were calculated.

2.1.2. Abattoir survey: F. hepatica determination in blood, stool, bile and condemned livers from slaughtered cattle

The surveys consisted in four weekly visits to the abattoir of Ponte Leccia between July and August 2020, being at that time the major cattle abattoir in all of Corsica. Whenever possible, samples of blood, stool and bile were collected from slaughtered animals. In addition, we were allowed to inspect (although we were not allowed to completely and thoroughly cut and dissect the livers) only the condemned livers with traces of distomatosis (infected by the liver flukes *F. hepatica* or *Dicrocoelium dendriticum*) observed in the abattoir sanitary inspection.

All collected samples were tagged with their bovine host ID in order to retrieve data at the host individual level (code of each animal, age, weight, race, name of the farmer and the municipality). One-hundred and fifty animals from 65 farms (representing 6.5% of cattle farms in Corsica (Serpentini et al., 2017)), mostly of mixed-breed (124), but also Corsican breed (22), Aubrac (2) and Limousine (2), were received at the abattoir. Most (93%) slaughtered cattle were <2-years-old with 37% younger than one-year-old. Overall mean age was 1.1 years (range 0.2–18 years).

2.1.2.1. Serological determination. Up to 10 mL of blood were individually collected from 146 animals (technical issues prevented us from collecting blood from the four remaining animals) into 15 mL tubes by post-mortem venepuncture at the moment of slaughter, and kept on ice until processing (for 4–5 h). Blood samples were centrifuged for 10 min at 800 xg, 4 °C to collect the serum that was further stored at −20 °C. Samples were screened by the commercial ELISA test SVANOVIR® *F. hepatica*-Ab accordingly to the manufacturer's instructions to investigate for the presence of anti-*F. hepatica* antibodies. All ELISA results were expressed as ODR (see section 2.1.1) and a cut-off value of ODR > 0.4 was used to discriminate positive from negative samples.

2.1.2.2. Copro-antigen determination. Up to 40 mL of stool were collected from the distal portion of the large intestine of slaughtered cattle using a scalpel. Stool samples were placed in 50 mL tubes maintained on ice until final storage at −20 °C. A total of 127 stool samples were screened to detect *F. hepatica* coproantigen using the commercial test BIO K201 ELISA (Bio XDiagnostics, Belgium) and the manufacturer's instructions. As indicated by the manufacturer, all ELISA results were expressed as Value = Δ ODsample * 100/Δ ODpositive control and the cut-off values indicated by the manufacturer for each batch of product were used to discriminate positive from negative samples.

2.1.2.3. Bile examination for egg screening. Up to 200 mL of bile were collected from the gall bladder of 139 slaughtered bovines into 200 mL clean flasks. Once in the laboratory, the content was allowed to sediment overnight at 4 °C. Further processing involved up to five cycles of washing with dechlorinated water for clarification, and sedimentation for collecting the eggs (see Mazeri et al. (2016)). The presence of both *F. hepatica* and *D. dendriticum* was verified by visualization of the eggs in the sediment under a stereoscope.

2.1.2.4. Condemned liver examination. Condemned livers with traces of distomatosis from the sanitary inspections by the abattoir (European guidelines; Regulation 2017/625) were examined through several incisions made with a knife. Manual pressing and squeezing were also exerted around the liver ducts towards the incisions to retrieve the flukes as we were not allowed to thoroughly dissect and slice up the livers. Obtained individuals of *F. hepatica* were saved in 50 mL tubes containing temperate saline solution (NaCl 0.8%) and properly identified with the code of the animal and transported alive to the laboratory. The number of collected flukes from each liver and number of co-infected livers were noted.

2.1.2.5. Data analyses. Overall and per-age interval (>1 year-old and < 1 year-old), rates of infection with 95% confidence intervals derived from the application of each method to the different samples were estimated as the percentage of positive samples. Test of proportions were used to compare infection rates between methods in Statistica v.12 (StatSoft. Inc., Tulsa, OK, USA 2014). Positive (by either previous serological and/or abattoir survey – see sections 2.1.1 and 2.1.2) and negative municipalities were mapped using MapInfo v.15 (Pitney Bowes Software Inc., New York, USA, 2015) for a spatial account of *F. hepatica* infection.

2.2. The intermediate hosts: Ecological and parasitological studies

2.2.1. Snail sampling and ecology

We surveyed 84 freshwater sites in Corsica in 2020. Freshwater snails were sampled during 15 min, keeping the sampling effort to a

Table 1
Details of the different PCR methods applied in the present study. dNTPs: deoxynucleosides triphosphate, BSA: bovine serum albumin.

PCR	Gene Target	Approach	Set of primer(s) – 5′3’	PCR mix conditions	Reference
Multiplex PCR	nuclear microsatellites	Molecular discrimination of *Galba* spp.	**Lc34** (f) GTCACTACT GCTTGTCTCA GC **Lc34(r)** AAAAGACTT TAACCCTTA CCACCC **Ls23** (f) AARGACCCA GTGGGGAAG Ls23(r) TGGGGAAGG TTCAATTGTT T **Lt37** (f) GTCCAGTCTT TGTATGTC **Lt37(r)** GTTAAGTAC CCAACTTCTT C	**Mix:** 5 μL of Taq PCR Master Mix Kit (Qiagen), 1 μL of the primer mix, and 50–100 ng of DNA, and water up to 10 μL of final volume. **Cycling:** 95 °C for 15 min; 35 cycles of: 30s at 94 °C, 90s at 52 °C, 1 min at 72 °C; extension for 30 min at 60 °C.	Alda et al. (2018)
End-point PCR	*coi*	Molecular discrimination within the Amphipepleinae clade	**LCO1490** GGTCAACAACTCATAAAGATATTGG **HCO2198** TAAACTTCAGGGTGACCAAAAAATCA	**Mix:** 1× GO buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs mix, 1 μM of each primer, 0.01% BSA, 1 U GoTaq® G2 Hot Start Polymerase (Promega), 1 μL of template DNA (sample diluted 1:5; *v*/v), and water up to 50 μL of final volume **Cycling:** 95 °C for 2 min; 35 cycles of: 40 s at 95 °C, 1 min at 50 °C, 1 min at 72 °C, extension for 10 min at 72 °C.	PCR; Vinarski et al. (2011) Primers; Folmer et al. (1994)
Multiplex PCR	*F. hepatica its-2*; Lymnaeidae *18S* (internal control)	Detection of *F. hepatica* in lymnaeid snails	**DSFf** ATATTGCGGCCATGGGTTAG **DSJ3** CCAATGACAAAGTGACAGCG **GcubF** GGGGAAGTATGGTTGCAAAGC **GcubR** CCCCAATCCCTAGCACGAAG	**Mix:** 1× GO buffer, 2.5 mM $MgCl_2$, 200 μM dNTPs mix, 1.2 μM of DSFf and DSJ3 primers, 0.4 μM of GcubF and GcubR primers, 0.01% BSA (if *P. columella* or *P. peregra* samples), 1 μL of DNA (diluted 1:5; v/v if *P. columella* or *P. peregra* samples), 1 U GoTaq® G2 Hot Start Polymerase (Promega), and water up to 25 μL of final volume **Cycling:** 95 °C for 5 min; 35 cycles of: 94 °C for 40 s, 60 °C for 60 s, 72 °C for 60 s, extension at 72 °C for 10 min.	Alba et al. (2015)
End-point PCR	Trematoda *16 S*	Detection of trematodes in lymnaeid snails	**Trem-16S-F1** GACGGAAAGACCCCRAGA **Trem-16S-R2** CRCCGGTYTTAACTCARYTCAT	**Mix:** 1× GO buffer, 1.25 mM $MgCl_2$, 200 μM dNTPs mix, 0.3 μM of each primer, 0.01% BSA, 1.25 U GoTaq® G2 Hot Start Polymerase (Promega), 1 μL of template DNA, and water up to 30 μL of final volume. **Cycling:** 94 °C for 3 min, 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s, extension at 72 °C for 5 min	Douchet et al. (2022)

minimum to further estimate the abundance per snail species (snails/15′; Perera (1996)), using 1 mm pore-size sieve and soft forceps. Collected snails were saved in 50 mL tubes and carried alive to the laboratory for identification and counting. At each site, geographic coordinates and altitude (GPS, Garmin), water temperature and pH (Hanna instruments), habitat type, hydrodynamic characterization (lentic/lotic), maximum depth and aquatic vegetation coverage were recorded.

2.2.2. Molecular identification of lymnaeid snail species

As cryptic species are known to exist in the family Lymnaeidae (Alda et al., 2018), lymnaeid species of the genus *Galba* and of the subfamily Amphipepleinae (species formerly within the genus *Radix*), were molecularly confirmed. Briefly, the shell of all individuals was carefully removed and a small piece of tissue from the foot was used for DNA extraction in a 100 μL of 5% Chelex® (Bio-rad) solution with 2.5 μL of proteinase K (Alba et al., 2015). DNA was further stored at −20 °C. DNA from individuals morphologically identified as *Galba* spp. (Lymnaeinae) were molecularly discriminated following the multiplex PCR by Alda et al. (2018).

Snails morphologically assigned to the Amphipepleinae clade were identified by amplification and sequencing of the *coi* gene (see Vinarski et al. (2011)) using the primers described in Folmer et al. (1994). Amplicons were sent and sequenced at the GenoScreen platform (Lille, France) using the LCO1490 primer. The resulting sequences were analysed using Mega v.5.0 (Tamura et al., 2011) and were further deposited in GenBank (accession numbers: ON792318, ON792319). Briefly, multiple alignments were performed with the Clustal algorithm against sequences of *P. peregra, A. balthica, A. auricularia, A. lagotis* revised and annotated by Aksenova et al. (2018), as it constitutes the largest and most inclusive study concerning Amphipepleinae. Sequences were manually trimmed and used to construct a Neighbour Joining tree with the Jukes-Cantor model and 1000 of bootstrap replicates. Details of the PCR assays are listed in Table 1.

2.2.3. Ecological analyses: Distribution and habitat preferences

We estimated the frequency of habitat types for each species of lymnaeid and the spatial distribution was mapped as well as for the rest of the occurring freshwater snails. Alpha diversity of freshwater snail species per site was estimated using Simpson's index (Simpson, 1949) and a multivariate canonical correspondence analysis (Ter-Braak and Verdonschot, 1995) was used to explore possible associations of species abundances and the abiotic and biotic factors.

2.2.4. Detection of F. hepatica in lymnaeid snails

Prior to DNA extraction, all lymnaeid snails were carefully dissected (Caron et al., 2008) to determine their trematode infection status. A multiplex PCR consisting on the amplification of a 340 bp species-specific amplicon of *F. hepatica* (ITS-2), and of a rDNA 18S fragment conserved to the family Lymnaeidae (451 pb), as internal control of the reaction per sample (Alba et al., 2015), was applied to the extracted DNA from all lymnaeid individuals for screening of the presence of *F. hepatica* (see Table 1). Positive controls consisted of spiked samples of 100 μg of *G. truncatula* DNA with 50, 10 or 5 μg of *F. hepatica* DNA, while water instead of DNA template was used as negative control. Snails positive to *F. hepatica* DNA per site per species were expressed as the percentage of snails showing an amplicon of the same size as the *F. hepatica* control in addition to the band corresponding to the internal control.

In addition, as the presence of other (unidentified) trematodes besides *Fasciola* was observed when dissecting lymnaeid snails, all collected individuals from the given site were further screened by PCR using a set of primers designed to amplify around 220 bp of a specific region within the *16S* rDNA gene of Trematoda (Douchet et al., 2022). Further details on the PCR conditions are listed in Table 1. DNA from adult *F. hepatica* was used as positive control, whereas two negative controls were also included: one consisting of DNA from non-infected lab-reared snail, and another where water instead of DNA template was added to the mix. Obtained amplicons were further sequenced by the GenoScreen platform (Lille, France) using the forward primer, blasted-x against the NCBI non-redundant databases to search for sequence identity and deposited in GenBank (accession numbers: ON814774-ON814777).

2.3. The environment: Exploring husbandry practices and environmental risks through a questionnaire

We administered a questionnaire to 62 Corsican farmers (representing 6% of specialized cattle and sheep farms in Corsica (Serpentini et al., 2017)). The questionnaire was amended and adapted from Kelly et al. (2016) and consisted of 30 questions distributed in 6 thematic blocks: i) farmer information (including the number and species of livestock), ii) knowledge about fasciolosis (including the most noticeable signs and symptoms of the infection and questions about recent reports in the given farm), iii) anthelmintic control routine (including the type/frequency of antiparasitic drugs), iv) grazing, v) transhumance (questions about the presence of flood-prone zones within pastures lands, the frequency and duration of both practices and the presence of stagnant natural waterbodies, and vi) the geographical scale relative to livestock trading at the given farm. The survey included farms devoted exclusively to bovine or ovine rearing as well as mixed farms, where both species are raised in sympatry. Data was summarized using descriptive statistics (percentages, median, mean, etc.) as epidemiological information on the circulation of *F. hepatica* within the animals (either by the serological or the abattoir screening) was only available for a limited number of farms.

2.4. Ethics statements

To carry out this study and to access biological material in the slaughterhouse, agreement No. 2020–064 was drawn up between the National Center for Scientific Research (CNRS) / University of Corsica (UCPP) and the *Syndicat Mixte de l'Abbatage en Corse* (SMAC; Collective Slaughtering Syndicate, Corsica). The SMAC is an agency that is mandated for the public service of slaughtering animals in Corsica. Anonymity in all procedures involving the screening of *F. hepatica* infection in livestock was always respected in accordance

with the agreement. The farmers' agreement to the take part in interviews and/or to allow visits to the farm was always obtained through an informed consent document. Likewise, during the abattoir surveys, notices were posted to inform users about the study taking place.

3. Results

3.1. The definitive host: Significant circulation of liver flukes in Corsican livestock

Although limited by the screening of pools of samples, the serological study performed at the farms (2018) showed a high percentage of positive batches of sera in both cattle 80% (270/338) and sheep 73% (268/367) resulting in 90% (251/279) of the sampled farms with at least one batch testing positive for anti-*F. hepatica* antibodies (92% of cattle farms and 89% of sheep farms). Estimated seroprevalence in cattle was 64% (96/150; 95% CI: 56–66%) and 44% in sheep (101/230; 95% CI: 37–51%) when sera were randomly and individually screened.

In the abattoir survey (2020), only two livers (2/150 = 1.3%; 95% CI: 0.06–5.04%) were rejected specifically due to *F. hepatica* by the official sanitary inspection. Both livers showed extensive fibrosis and liver damage from which 12 and 81 adult flukes were retrieved respectively. In contrast, overall infection rates were higher on the basis of the other approaches used (pairwise comparison with proportion tests; $P < 0.0001$; see Table 2 for details). The highest seroprevalence was recorded in animals older than 1-year-old. No significant differences were found among the results of the different methods in <1-year-old livestock, possibly associated with the fact that most animals had lived through only one transmission cycle. Significant differences were obtained between serology and the direct methods (eggs and copro-antigen determination) when applied to older animals (> 1-year-old). However, similar infection rates were recorded by the two latter methods (Table 2). Animals found positive by at least one method gave an overall prevalence of 75% (49/65) of the sampled farms.

Our survey based on egg detection in the bile showed higher infection rates for the lancet fluke *D. dendriticum* than the routine veterinary inspection. The presence of *D. dendriticum* eggs was confirmed in 96 out 139 analysed bile samples (69%; 95% CI: 61–77%), whilst only 18 livers (12%; 95% CI: 7.3–18%) were condemned by the abattoir due to this trematode. Co-infection with both liver flukes was patent in one of the *F. hepatica*-infected livers when inspected, as well as in 22 other animals according to bile examination (23/139 = 17%; 95% CI: 11–24%).

Fig. 1 shows a ubiquitous distribution of *F. hepatica*-positive animals at the municipality level throughout the island (2018–2020) ranging from littoral to mountain landscapes. Overall, 134 out of 149 based on serological survey municipalities and 36 out of 46 based on abattoir survey municipalities were positive whereas 20 were positive by both epidemiological studies.

3.2. The intermediate host: Ecology and infectious status of lymnaeid snails in Corsica

The malacological survey of 84 freshwater sites throughout Corsica (Fig. 2A) allowed the identification of 11 snail species (supplementary Fig. 1) and at least two unidentified bivalve species. The species found most frequently was *Physa acuta* (frequency = 0.35) followed by the New Zealand mud-snail *Potamopyrgus antipodarum* (frequency = 0.27), and the planorbid limpet *Ancylus fluviatilis* (frequency = 0.27; see supplementary Fig. 1).

Concerning lymnaeid snails, three species were recorded at 18 sites (Fig. 2B) across a variety of habitat types (Fig. 2D). In particular, we report for the first time the presence of the American species *P. columella* in Mediterranean islands. This species was found in a lake (Figari) in the southernmost part of Corsica (Fig. 2B,D). Sequencing of the Amphipepleinae species corroborated the initial morphological identification of *P. peregra* (see Fig. 2C for details on the shell) as they grouped within this clade with a bootstrap of 85 (Fig. 2E). The sequenced populations of *P. peregra* occurring in southern Corsica (Cavu and Bicchisano; lowlands populations) clustered together but were segregated from others of the group, including two populations from northern Corsica (occurring at Ballicione and Corte, above 400 m). The amphibious lymnaeid *G. truncatula* was PCR-confirmed at the nine sites where *Galba*-like snails were collected. This species was found in sympatry with *P. peregra* in the stream Forcalello (see Fig. 2B for details on the

Table 2
Infection rates and statistical differences between diagnostic methods to detect *F. hepatica* infection in slaughtered cattle from Corsica (year of study: 2020). Ab: antibodies, Ag: antigen, CI: confidence intervals, NS: non-significant, y-o: year old.

	Abattoir inspection	Serum (Ab) ELISA	Copro-Ag ELISA	Bile examination (eggs detection)
Infection rates				
Overall % (95% CI)	1.34 (0.06–5.0)	46 (38–55)	15 (10–22)	19 (13–26)
<1 y-o % (95% CI)	0	34 (21–53)	22 (11–40)	22 (12–37)
>1 y-o % (95% CI)	2.35 (0.29–8.5)	54 (40–72)	10 (4–20)	16 (9–28)
<1 y-o vs. >1 y-o	NS	0.01	NS	NS
Statistical significance				
Abattoir inspection	–	0.0001	0.0001	0.0001
Serum (Ab) ELISA	0.0001/0.0001	–	0.001	0.001
Copro-Ag ELISA	0.0001/0.0001	NS/0.0001	–	NS
Bile examination (eggs)	0.0001/0.0001	NS/0.0001	NS/NS	–

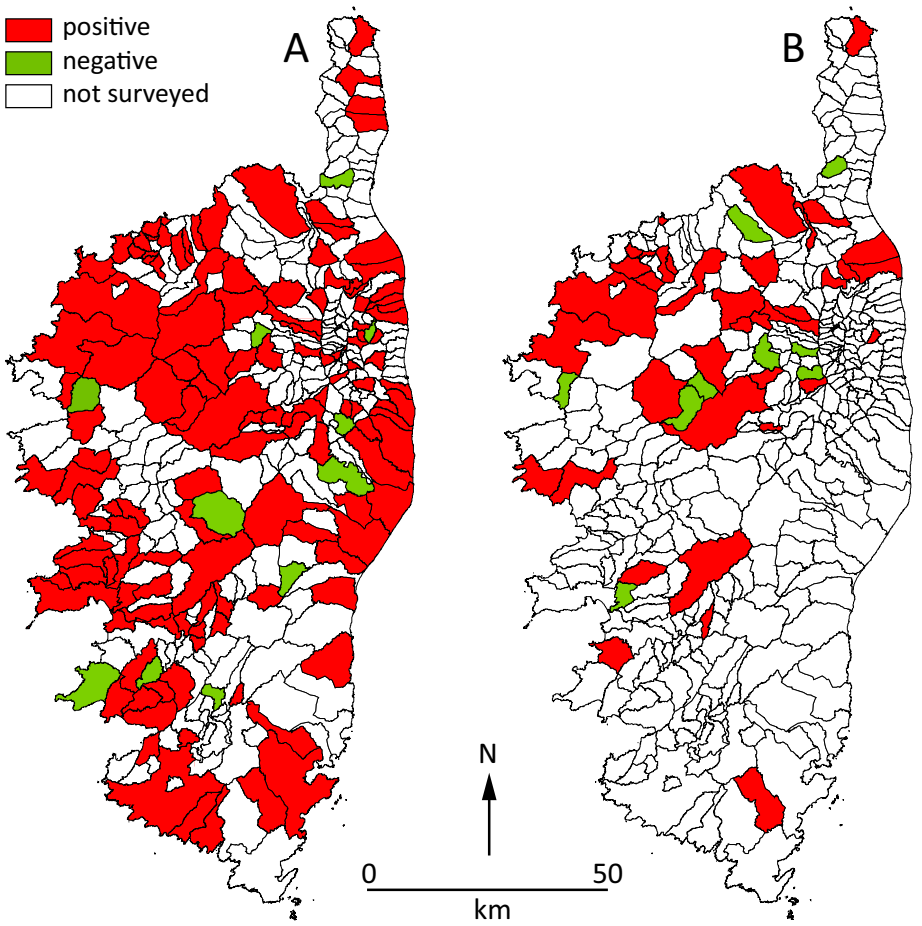


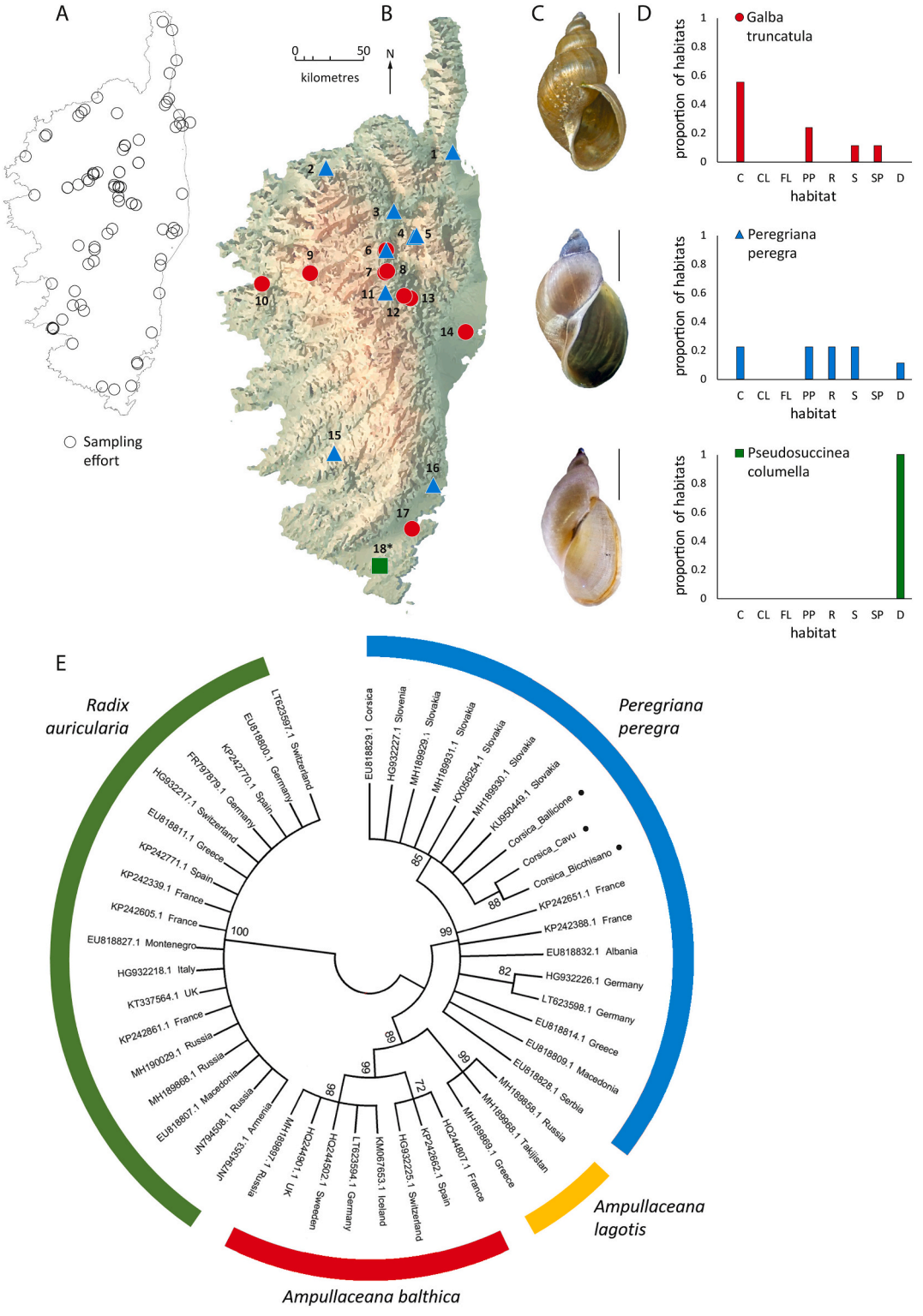
Fig. 1. Distribution of positive and negative municipalities of Corsica with regard to *Fasciola hepatica*–infected livestock according to either previous within-farm serological (A) in 2018 and abattoir (B) surveys in 2020.

localization of the sites).

All 253 lymnaeid snails (125 *G. truncatula,* 111 *P. peregra,* 17 *P. columella*) collected in Corsica were dissected to detect the presence of larval trematodes. All lymnaeid snails were negative for *F. hepatica* infection by dissection but were further screened by the multiplex PCR. On the basis of the negative co-amplification of a Lymnaeidae-conserved 18S fragment used as internal control per PCR reaction, inhibition of the amplification reaction was identified in 28 lymnaeid snails (mostly *P. peregra* and *P. columella*), even after the improvement of the reaction conditions (dilution of DNA, addition of BSA). Two *P. peregra* snails, one from Bevinco river (1/8 = 12%, 95% CI: 0.32% - 53%) and one from Ballicione stream (1/16 = 6%; 95% CI: 0.16% - 30%) showed a pale band of the expected size of *F. hepatica*, possibly indicating a very recent exposure to the parasite or the presence of DNA remnants of the parasite from past infections (Fig. 3A).

In addition, several larvae from an unidentified trematode were observed by snail dissection in four *G. truncatula* from a ditch in T50 Venaco. All 48 individuals collected from this site were then screened by an end-point PCR for amplification and further sequencing of the 16S of Trematoda. All four dissection-positive snails along with five other individuals amplified the expected fragment of Trematoda 16S (19%; Fig. 3B). The obtained sequences corresponding to four different genotypes presented 92% sequence identity with *Plagiorchis maculosus* (E value $<10^{-69}$). Further investigations are thus needed for further taxonomical discrimination.

Concerning habitat type, an overall preference for lentic ecosystems was patent in the three lymnaeid species (13 out 18 sites; Fig. 2D). In particular, most *G. truncatula* records were found in canals/ditches and stagnant ponds (8/9), whereas *P. peregra* was found in open or connected water systems of rivers and streams (5/9) or in four other different habitats featuring closed ecosystems (Fig. 2D). The canonical analysis explained 74% of the variance in its two first axes and showed a positive association of the presence and abundance of *G. truncatula* and, to a lesser extent, of *P. peregra* with increasing pH and altitude. A negative association was found, however, between these two species and water temperature and conductivity (Fig. 4).



(caption on next page)

Fig. 2. (**A**) Distribution of all sampled freshwater sites accounting for sampling effort. (**B**) Sites positive to lymnaeid snail species in Corsica: 1) Bevinco, 2) Codole, 3) Ballicione, 4) Morachini, 5) San Lorenzo, 6) Forcalello, 7) Mare Bois de St Jean, 8) Canal Bois de St Jean, 9) Col di Vergio, 10) Porto, 11) T20 Venaco, 12) T50 Venaco, 13) Odarc-Pont Altini, 14) T50 Vaccaja, 15) Bicchisano, 16) Cavu, 17) Porto Vecchio, 18) Figari. **(C)** Shells and **(D)** number of records of each lymnaeid species found per type of habitat. **(E)** Neighbour-joining tree constructed with sequences of the subfamily Amphipepleinae and three sequences (black pointed sequences) from three Corsican sites positive to this clade. (*) represents newly recorded lymnaeid species in Corsica. C: Canal/Ditch, CL: coastal lagoon, FL: flooded lands, PP: permanent pond, R: river, S: stream, SP: seasonal pond, D: dam/lake. Scale: 5 mm.

3.3. The environment: Livestock management practices among Corsican farmers

Table 3 summarizes the results from the questionnaire administered to 62 farmers inhabiting all the geographical regions of Corsica. Most farmers referred to have known or heard about fasciolosis irrespective of the type of livestock owned. Among the signs and symptoms that the interviewees associated with *F. hepatica*, the ones most frequently referred to were neck swelling or bottle jaw, and weight loss. Around 1/3 of the interviewees indicated having observed the identified clinical signs in animals from their herds, with farmers from cattle and mixed farms even referring to having suffered significant fasciolosis outbreaks during the last five years. Liver fluke infection from sold or slaughtered animals was confirmed by bovine farmers, whether in mixed or cattle-exclusive farms (Table 3); they were used mostly for meat production (ovine livestock is mostly used in Corsica to produce dairy products with only lambs, usually <6 months old, being sold or slaughtered for meat production).

Concerning treatment, 14 anthelminthic drugs were referred to as being used by the interviewees while only five are either specific to or have a certain effect against *F. hepatica*, whether against adult flukes (albendazole, netobimin, oxyclozanide, clorsulon) or against both adult flukes and immature stages (closantel; see Table 3). Significantly, 13 farmers (21%) did not mention flukicide drugs within their treatment scheme. Sheep farmers mostly reported a generalized use of the broad-spectrum anti-parasitic drugs albendazole and netobimin, whereas cattle farmers used specific flukicides, with clorsulon as the most frequent drug combined with ivermectin (clorsulon + ivermectin). The other two specific anti-*F. hepatica* treatments were mentioned by three cattle farmers who acknowledged having significant difficulties with fasciolosis. One of these farmers treated livestock with closantel (in February) after two treatments with clorsulon + ivermectin (June and October). Noteworthily, these farms resulted positive to *F. hepatica* in both the serological and abattoir surveys, signifying a constant circulation of the parasite within these herds. The frequency and timing of the flukicide treatments vary among farms with differences observed even when using the same drug, particularly when applying clorsulon + ivermectin. The majority of cattle farmers applying the combination of ivermectine + clorsulon treated their animals only once a year, although referring indiscriminately to up to eight different months and three seasons (winter, spring and autumn) when asked for the timing. Conversely, most sheep farmers treated twice a year mostly animals older than 1-year-old, at the beginning of both summer and autumn. In the case of farmers treating once a year, the most frequent period referred to was the beginning of autumn. (Table 3).

Cattle and sheep herding is a generalized practice among all interviewed farmers, with most of the animals grazing freely and having access to natural freshwater sites. The presence of lymnaeid-prone ecosystems in Corsica such as ponds, puddles and streams were frequently referred to occurring within grazing areas. Periodic flooding of pasture lands, mostly during late autumn and early winter, was also indicated by around one third of the interviewees, and noted to be particularly frequent in the surveyed cattle farms. Transhumance, a traditional husbandry practice which consists in transporting the herds during summer to grazing areas outside the farms, usually located in the uplands, and leaving them to freely forage and graze, was mentioned by one third of cattle farmers and by more than half of the mixed farmers, mostly associated with the management of bovine livestock. In general, the shorter mean distance between the farm and the transhumance area and the longer mean time of the transhumance period were related to cattle farms. Concerning trading practices, most interviewees indicated that they buy and sell within Corsica, although some indicated that they even acquire cattle from metropolitan France (Table 3).

4. Discussion

4.1. Fasciolosis in Corsica: An overlooked issue

4.1.1. On the definitive host

Studies of fasciolosis in Corsica are sparse, scarce, and probably outdated as there has been little incidence of reported human cases (see WHO (1995)). Concerning animal fasciolosis, although there have been no specific studies, references dating from 60 years ago referred to high prevalence in both sheep and cattle during slaughtering and alerted to the potential problem of *F. hepatica* infection in the livestock of Corsica (Gretillat, 1963). Nevertheless, nowadays infection in livestock is assumed to be of little concern in Corsica.

Without periodic specific surveys using sensitive methods, the epidemiological status of fasciolosis in livestock is mostly dependent on isolated acute outbreaks or on sanitary inspections at the abattoirs. The latter is biased towards underestimating the impact of fasciolosis, as has been shown in the present study. Although differences are expected between countries and potentially between abattoirs, the lower sensitivity of liver inspection compared to other methods such as serological screening and/or bile examination from the gall bladder has been reported, particularly when performed by the meat inspectors at the abattoir (see Rapsch et al. (2006) in Switzerland, Mazeri et al. (2016) in United Kingdom). Liver inspections need to be thorough and to involve extensive cutting and slicing of the organ to achieve greater sensitivity, especially if parasitic intensities are low. However, such procedures are time-consuming, and impractical for routine application at abattoirs (Mazeri et al., 2016; Rapsch et al., 2006). In this sense, as the majority of slaughtered cattle in Corsica are under 2 years old, parasite load and liver fibrosis could be low enough to be overlooked by the

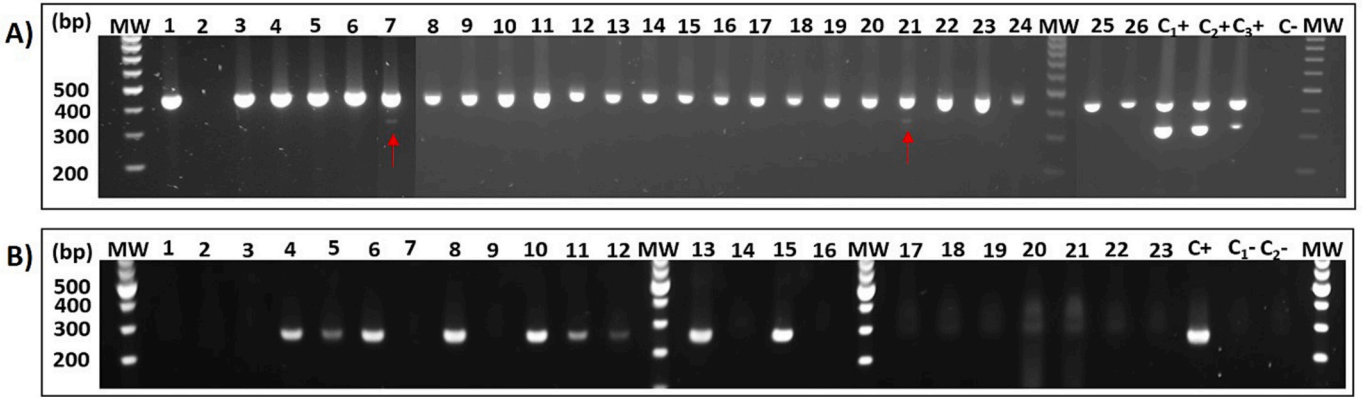


Fig. 3. **(A)** Agarose gel (2%) showing results of *F. hepatica*-specific multiplex PCR where two *P. peregra* showed the 340 bp amplicon corresponding to *F. hepatica* ITS2 (indicated by red arrows). Non-inhibited samples show the internal control amplicon corresponding to Lymnaeidae 18S (451 pb; Alba et al., 2015). C+: positive control consisting on spiked samples *G. truncatula* DNA (100 µg) / *F. hepatica* DNA (50, 10 or 5 µg), C-: negative control where water instead of DNA template was added. **(B)** Agarose gel (2%) showing the 240 bp amplicon of the *coi* gene for Trematoda amplified in *G. truncatula*. MW: Molecular weight marker GeneRulerTM 100 bp DNA ladder. C^{+}: *F. hepatica* DNA, C_1^{-}: negative control consisting of DNA from a non-infected lab reared snail, C_2-: negative control where water instead of DNA template was added. MW: Molecular weight marker GeneRulerTM 100 bp DNA ladder. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

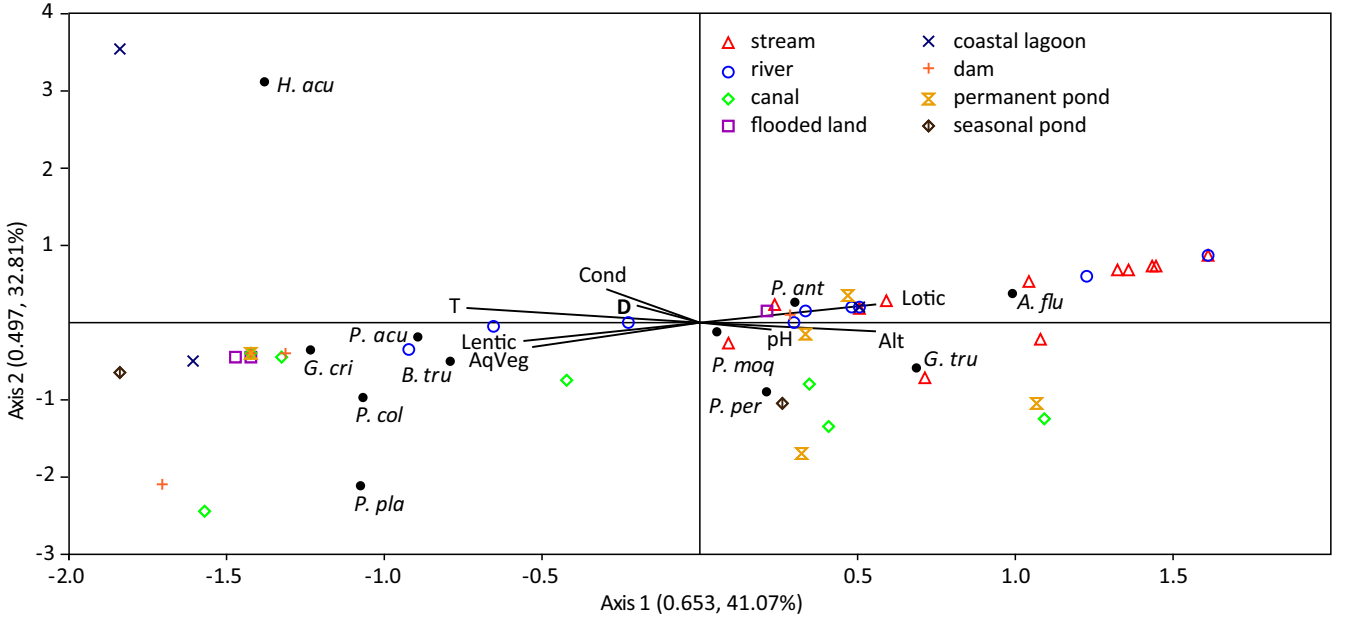


Fig. 4. Scatterplot of the first two dimensions of the canonical correspondence analysis showing ecological variables and species abundances in sampled sites across Corsica (overall explained variance: 73.8%). AqVeg = coverage of aquatic vegetation, Alt = altitude, Cond = conductivity, D = diversity, T = temperature. Snails: *A.flu* = *Ancylus fluviatilis*, *B.tru* = *Bulinus truncatus*, *G. cri* = *Gyraulus crista*, *G.tru* = *Galba truncatula*, *H.acu* = *Hydrobia acuta*, *P.acu* = *Physa acuta*, *P.per* = *Peregriana peregra*, *P.ant* = *Potamopyrgus antipodarum, P.col* = *Pseudosuccinea columella*, *P.moq* = *Planorbis moquini, P. pla* = *Planorbis planorbis.*

Table 3
Summary of questionnaire data stratified by cattle, sheep and cattle/sheep mixed farms.

Farm factors	Cattle	Sheep	Mixed
Number of farms	29	23	10
Mean herd size (range)	67 (15–300)	253 (40–500)	Cattle: 44 (15–80) Sheep: 124 (30–250)
Median of years dedicated by the farmer to husbandry	30	22	33.5
Farmers referring to knowing *F. hepatica* and/or fasciolosis	93% (27/29)	78% (18/23)	80% (8/10)
Informed farmers correctly referring to at least one sign/symptoms of fasciolosis	74% (20/27)	67% (12/18)	88% (7/8)
Death of the animal	19% (5/27)	5% (1/18)	13% (1/8)
Bottle jaw/neck swelling	59% (16/27)	50% (9/18)	63% (5/8)
Reduced fertility	0	0	0
Weight loss	37% (10/27)	22% (4/18)	38% (3/8)
Weakness	15% (4/27)	5% (1/18)	0
Pale mucosa of eyes and gingiva (anemy)	0	5% (1/18)	0
Reduced milk production	0	5% (1/18)	0
Diarrhoea	4% (1/27)	0	13% (1/8)
Farmers referring to having observed animals with at least one sign/symptoms of fasciolosis in the last 5 years	33% (9/27)	33% (6/18)	38% (3/8)
Farmers referring to having had mortality due to fasciolosis in the last 5 years	20% (6/29)	0	33% (3/10)
Farmers referring to having been informed of sold or slaughtered animals infected with *F. hepatica*	48% (14/29)	17% (4/23)	40% (4/10)
Overall use of flukicide treatment	**83% (24/29)**	**70% (16/23)**	**90% (9/10)**
Flukicide treatment: **Albendazole**	**0**	**50% (8/16)**	**11% (1/9) – sheep**
Age of treated animals: >1 year old	0	100% (8/8)	100% (1/1)
Frequency and timing per year: twice –Jun/Jul & Sep/Oct	0	50% (4/8)	100% (1/1)
Frequency and timing per year: once: Sep/Oct	0	50% (4/8)	0
Flukicide treatment: **Netobimin**	**0**	**44% (7/16)**	**11% (1/9) – sheep**
Age of treated animals: all ages	0	14% (1/7)	0
Age of treated animals: >1 year old	0	86% (6/7)	100% (1/1)
Frequency and timing per year: twice – Jun/Jul & Sep/Oct	0	71% (5/7)	0
Frequency and timing per year: once – Sep/Oct	0	29% (2/7)	100% (1/1)
Flukicide treatment: **Clorsulon**	**92% (22/24)**	**6.3% (1/16)**	**89% (8/9)**
Age of treated animals: all ages	100% (22/22)	100% (1/1)	100% (8/8)
Frequency and timing per year: once – Dec/Feb	32% (7/22)	0	63% (5/8)
Frequency and timing per year: once – Mar/May	9% (2/22)	100% (1/1)	25% (2/8)
Frequency and timing per year: once – Oct/Nov	23% (5/22)	0	0
Frequency and timing per year: twice –Dec/Feb & Sep/Nov	13% (3/22)	0	0
Frequency and timing per year: twice –Apr/Jul & Sep/Nov	23% (5/22)	0	12% (1/8)
Flukicide treatment: **Closantel**	**8% (2/24)**	**0**	**0**
Age of treated animals: all ages	100% (2/2)	0	0
Frequency and timing per year: once – Oct/Nov	50% (1/2)	0	0
Frequency and timing per year: once – Feb	50% (1/2)	0	0
Flukicide treatment: **Oxyclozanide**	**5% (1/24)**	**0**	**0**
Age of treated animals: all ages	100% (1/1)	0	0
Fequency and timing per year: twice - Jun & Oct	100% (1/1)	0	0
Pastoralism	**100% (29/29)**	**100% (23/23)**	**100% (10/10)**
Free access to natural water sources	100% (29/29)	78% (18/23)	90% (9/10)
Flooded terrain	41% (12/29)	33% (6/18)	22% (2/9)
Pond (temporal and permanent)	28% (8/29)	39% (7/18)	11% (1/9)
Canal/ditch	24% (7/29)	6% (1/18)	11% (1/9)
River	55% (16/29)	50% (9/18)	44% (4/9)
Stream	66% (19/29)	44% (8/18)	44% (4/9)
Lake	7% (2/29)	6% (1/18)	0
Puddles	28% (8/29)	22% (4/18)	22% (2/9)
Transhumance	**31% (9/29)**	**13% (3/23)**	**60% (6/10)**
Mean distance from the farm to the transhumance zone	15.3 km	25 km	17.5 km
Mean time for transhumance	6 months	4 months	5.3 months
Trading of livestock	**72% (21/29)**	**78% (18/23)**	**80% (8/10)**
Purchase within the same municipality	21% (4/19)	13% (2/15)	0
Purchase within Corsica	74% (14/19)	87% (13/15)	100% (8/8)
Purchase in metropolitan France	21% (4/19)	0	13% (1/8)
Selling within the same municipality	33% (4/12)	14% (1/7)	50% (1/2)
Selling within Corsica	67% (8/12)	86% (6/7)	50% (1/2)

liver inspection at the abattoir as younger animals may have lower prevalence/intensity due to less exposure. In addition, the higher prevalence and intensity of the lancet liver fluke (*D. dendriticum*) in Corsican cattle reported here as elsewhere in metropolitan France (Vázquez et al., 2021) could also influence detection by providing an easier and faster diagnosis for co-parasitized livers. All these

factors could help to explain the major differences between liver inspections and the other screening methods observed in the present investigation.

Serological screening may allow very early detection of infections as well as past exposures to the parasite (Alvarez Rojas et al., 2014), although the latter effect is diminished in younger animals which are potentially exposed to fewer transmission periods. Due to this, parasite prevalence could be overestimated by serological screening, particularly in older animals, but it could be useful for estimating the circulation of the parasite (Charlier et al., 2009). Cross-reactivity of circulating antibodies raised against others worms can also occur when applying serological methods, giving false positive results (Alvarez Rojas et al., 2014). However, field assessment of the Svanova kit for anti-*Fasciola* antibodies in different European settings accounted for relatively high specificity (ranging from 83 to 96%; (Charlier et al., 2014, Munita et al., 2019)). The commercial copro-ELISA is a considerable modification of the original immuno-enzymatic test based on the MM3 monoclonal antibody (see Mezo et al. (2004)). Although in the present study no significant differences were attained between copro-antigen and egg detection, the performance of the former test in the field has been poorly evaluated, especially in cattle, and lower sensitivity has been recorded if using the indicated cut-off value when compared to other methods (see Mazeri et al. (2016), Palmer et al. (2014)). The examination of bile for the presence of *F. hepatica* eggs renders trustworthy results in terms of prevalence. However, there is always a possibility of gall bladder egg sequestration in animals where infection has been successfully treated or of early infections being undetected due to the sexual immaturity of the parasites, causing false negative results (Mazeri et al., 2016; Rapsch et al., 2006).

Epidemiological data in the previously performed and unpublished study presented here are limited by the large-scale screening of pooled blood instead of individual samples, and by the selection of samples from positive pools for individual animal screening. Given the inherent advantages and drawbacks of each method in relation to the biology of *F. hepatica* infection, a multi-screening approach as the one performed at the abattoir is appropriate for characterizing the epidemiological scenario of animal fasciolosis. However, the abattoir survey is limited by the small number of slaughtered animals. Nonetheless, by combining the two studies, there are sufficient grounds to depict the sustained circulation of *F. hepatica* throughout the island (see Fig. 1). Moreover, patent infection was also significant as observed by the presence of *F. hepatica* eggs in young cattle (<1 year-old) which, giving the age of the animals, indicates recent infections and highlights the permanence of the life cycle of *F. hepatica* in domestic animals in Corsica. Similar results were observed during serological screening in metropolitan France with around 90% of cattle farms testing positive for anti-*F. hepatica* antibodies and 20% of sampled animals showing patent infections through coprological screening (Bosquet et al., 2007).

4.1.2. On the intermediate host

Our malacological survey has updated the distribution of freshwater snail species in Corsica, emphasizing the occurrence of lymnaeid snails at all altitudes and most sampled habitat types (except brackish waters of coastal lagoons). Such ecological plasticity makes this family of snails ubiquitous in the field and thus supports its role as transmitter of parasitic diseases such as fasciolosis. Interestingly, the three lymnaeid species found differed in frequency and in habitat types with *G. truncatula* mostly occurring in stagnant /slow-flowing waters or anthropic sites, as reported elsewhere (de Kock et al., 2003; Hammami and Ayadi, 1999; Kleiman et al., 2007; Schweizer et al., 2007).

The canonical correspondence analysis revealed a positive association of *P. peregra* but mostly of *G. truncatula* with increasing altitudes, indicating their suitability for successfully inhabiting a predominantly mountainous island. Elsewhere, *G. truncatula* is highlighted as the main driver of fasciolosis transmission in the Andean Altiplano (Meunier et al., 2001) and in the Pyrenees (Roldán et al., 2020). This species is also acknowledged to be the main intermediate host of *F. hepatica* in Europe (see Vázquez et al. (2018)) and Corsican populations proved compatible with Corsican parasite isolates in the past (Oviedo et al., 1996). Likewise, our finding of *F. hepatica* DNA within *P. peregra* might indicate the potential for transmission in Corsica by this European snail, which is frequently found in rivers and streams, the two principal water bodies throughout the island. As no larval stages were observed, further investigations are needed to properly depict the role of this species in *F. hepatica* transmission and that being the case, to determine the natural prevalence of *F. hepatica* in snails on the island. Nonetheless, this result highlights the constant circulation of the parasite in the field in Corsica.

Significantly, the sighting, for the first time in Corsica, of the exotic species *P. columella* could impact even further fasciolosis transmission on the island. This species has achieved a significantly extensive rapid invasion with established populations outside its native North American range in South America and the Caribbean, Africa, Australia and the Pacific islands, and more recently, in continental Europe (see Lounnas et al. (2017)). These worldwide invasions have been mostly carried out by a highly invasive multilocus genotype that is also highly compatible with *F. hepatica* (Lounnas et al., 2017), and has facilitated parasite spill-backs and the transmission of *F. hepatica* (Alba et al., 2019; Dar et al., 2015; Prepelitchi et al., 2003) and *F. gigantica* (Grabner et al., 2014) in the invaded regions. Further studies are needed to explore and characterize the presence of this species in Corsica.

4.1.3. On the environment in terms of husbandry practices and risks

Lastly, as domestic animals are under human management, it is essential to examine husbandry practices in order to comprehensively understand the epidemiology of diseases such as fasciolosis and to pinpoint risky behaviours. Interestingly, although fasciolosis is acknowledged as a veterinary disease by most of the farmers interviewed, 13 do not mention using any flukicide treatment, which could indicate a low perception of the risk of transmission, and a disregard for the disease. This overall scenario could be fairly common among farmers and has in fact been reported elsewhere in Europe and other regions of the world; e.g. (Knubben-Schweizer et al., 2010; Opsal et al., 2021; Kelley et al., 2021). Concerning those using flukicides, the frequency and timing of the treatment are factors as important as the type of treatment itself to take into account to achieve proper control of transmission. A variable efficacy against mature flukes have been reported for netobimin in the past (Richards et al., 1987), while more recent studies in naturally-

infected sheep recorded low or no change in *F. hepatica* egg outputs (Sánchez-Andrade et al., 2001; Arias et al., 2009). Because in temperate regions transmission of *F. hepatica* follows a bi-seasonal pattern in summer and winter with prevalence in livestock increasing as the grazing season progresses (Bloemhoff et al., 2015; Munita et al., 2019), it is particularly recommended to treat animals in late autumn and/or in winter, to reduce not only the parasite load in livestock but also the contamination of pastures with *Fasciola* eggs (Fairweather et al., 2020). It is therefore possible to leave out some infected animals with migrating juveniles or immature parasites if, as observed herein, drugs active only against adult stages are applied early in the summer or early in the fall. Such an approach could significantly reduce the risk of acute fasciolosis outbreaks, particularly in sheep (to what is called "the economic threshold") but it is not optimal for interrupting parasite circulation (Fairweather et al., 2020). In the case of products of combined drugs, although they have practical advantages, they need to be used judiciously, particularly regarding when to treat, as the ideal time is likely to be different depending on the potential target parasites (Fairweather et al., 2020). This could relate to the variation observed in the present study concerning the use of ivermectin + clorsulon by cattle farmers. It is important to mention that several farms where flukicides were applied presented infected animals with patent infections, observed during the abattoir survey, which highlights the necessity for more holistic actions that consider not only the type, frequency and timing of the antiparasite treatment but also the contamination of the pastures and the occurrence of the intermediate host within the area.

Other management practices referred to by the farmers interviewed could favour the circulation of *F. hepatica* in livestock. As it is customary in Corsica, sheep and cattle graze freely on pasture lands most of which present uncontrolled natural water sources where lymnaeid snails can thrive and where active transmission foci can occur (see our malacological results). Feeding on flooded or wetland pastures vs. feeding on forage/dry-land crop residues, using unsafe water resources and prolonged grazing, have been pointed out as significant risk factors for *F. hepatica* infection to occur in livestock ((Khan et al., 2009, Suon et al., 2006, Takeuchi-Storm et al., 2017), for review see Sabourin et al., 2018). Furthermore, cattle seem to be sent to transhumance more frequently than sheep with >30% grazing freely during summer months mostly on the uplands where Corsican lymnaeid snails can be abundant (see section 3.2). In this regard, experimental studies on *G. truncatula* from France have shown a higher compatibility in highland vs. lowland populations to sustain *F. hepatica* (Vignoles et al., 2002). Livestock movements during transhumance could have an even deeper impact on transmission as it favours gene flow not only among domestic animals from different farms and regions if transhumance areas overlap, but also between domestic livestock, wild cattle (typical in Corsica) and wildlife, which could result in a higher parasitic plasticity. Vázquez et al. (2021) showed that susceptible wildlife (wild boar and coypu) in the lowlands of Camargue, Southern France plays a pivotal role in maintaining and dispersing *F. hepatica* in natural environments, and in homogenizing the metapopulation of the parasite throughout the area. In Corsica, in particular, black rats are acknowledged as a significant reservoir of *F. hepatica* with prevalence of up to 58% (Magnanou and Morand, 2006). Gene flow of *F. hepatica* throughout the island can be also assumed as most cattle trading remains within Corsica.

4.2. Comments regarding the eco-epidemiological scenario of fasciolosis in Corsica

The livestock industry in Corsica has experienced change in the last decades driven by a variety of economic pressures. Between 1970 and 2013, it underwent an overall reduction (almost a third) of the number of livestock smallholdings, but an increase in the number of breeding animals (sheep from 81,600 in 1970 to 91,900 in 2010; cattle from 21,900 in 1970 to 40,900 in 2010) and in the area devoted to grazing, given that extensive grazing remains the predominant productive strategy (Serpentini et al., 2017). All this ultimately implies an increase of livestock density and of land use, and thus an increased potential for the transmission of parasitic diseases such as fasciolosis (Alba et al., 2021). The diversity of the livestock has also changed with the local breeds of sheep, but in particular of cattle, decreasing in number in favour of mix-breed animals of continental origin (Serpentini et al., 2017). Although there have been no studies on the susceptibility to *F. hepatica* of the Corsican breed of bovines compared to other breeds, the introduction of new genotypes and the concomitant reduction of the local genetic pool might affect transmission.

Furthermore, with five lymnaeid species already reported over Corsica (Glöer, 2019), the recording on the island of one of the major hosts of *F. hepatica* worldwide, the species *P. columella*, is worth monitoring. Biological invasions usually have significant effect on disease dynamics and have had an influence in shaping the current epidemiology of fasciolosis (see (Alba et al., 2021, Vázquez et al., 2022)). They can be driven or boosted by human activities (see Sabourin et al. (2018)) and factors such as climate change weigh heavily in promoting or hindering the colonization and establishment of exotic species in new areas (Hulme, 2016). Corsica already exhibits evidence of the effect of the climate change including an increase of the mean annual temperature and of the number of hot days, a decline in the number of days of frost, and a clear trend in favour of continued warming (Serpentini et al., 2017). These conditions and trends could facilitate the invasion of *P. columella* and complicate the overall scenario of *F. hepatica* transmission in the warmer lowland areas within the island.

Fasciolosis dynamics are directly and ultimately linked to livestock management and cultural habits. Extensive grazing and transhumance practices occurring in the Corsican livestock industry could significantly promote *F. hepatica* transmission if they are not carefully controlled and supervised. Even appropriate practices such as fencing, desiccation of water bodies and pasture rotation may be little suited to preventing new infections and the contamination of grazing fields if the presence of intermediate hosts and the dynamics of the *F. hepatica* cycle are not taken into account (see Knubben-Schweizer et al. (2010), Knubben-Schweizer and Torgerson (2015)).

Beyond technical and knowledge-related issues, it is primarily the low perception of the disease and its risks that hinders proper control. This is particularly concerning when control measures rely mostly on the use of antiparasitic drugs. Anthelmintic treatment, as a general control practice in Corsica, is usually deployed by the veterinarians of each locality and is usually carried out without well-established diagnostic criteria. This is particularly important when using combination treatments, as observed here with the extended

used of ivermectin + clorsulon for treating cattle, and it should always involve the identification of which parasites are actually present before deciding when to treat (Fairweather et al., 2020). Acute fasciolosis causing mortality is most common in sheep when intensities are high, whereas cattle usually present a chronic infection. Factors such as the (suboptimal) use of flukicide treatment, the preferential slaughtering of young cattle and the resilience of bovines, particularly when the parasite loads are not very high, could stifle acknowledgment of the circulation of the parasite among the herds. To tackle this, more refined detection methods than the traditional abattoir inspections or mortality events could be introduced. In the case of the sanitary inspections at the abattoir, it is worth mentioning that besides issues of sensitivity, revealed in the present investigation regarding the detection of *F. hepatica*, since 2008 official reports have not differentiated between liver fluke species. Given the frequency and intensities of the lancet liver fluke in Corsican livestock, most reports reaching the farmers and veterinarians are assumed to be of *D. dendriticum*. Thus, herein and elsewhere (see Alba et al. (2021)), the deployment of specific epidemiological surveys and of the application of novel diagnostic techniques have proved essential in depicting the real impact of fasciolosis.

Lastly, it should be mentioned that, although we only analysed *F. hepatica* in livestock as definitive hosts, the circulation of the parasite in nature offers risks for the occurrence of human infection. The most recent reported cases of human fasciolosis in Corsica have come from the active screening of blood samples collected between 1984 and 1990; 18 out of 228 persons tested positive for anti-*F. hepatica* antibodies (WHO, 1995). However, the diagnosis of human fasciolosis could be also problematic and several factors contribute to a frequent sub-diagnosis of its incidence in human population unless looked for (Toet et al., 2014).

5. Conclusions

In the present study, we show that animal fasciolosis in Corsica is characterised by a significant circulation and a favourable epidemiological scenario for transmission to occur. Infection by *F. hepatica* was patent in livestock and DNA of the parasite was amplified in lymnaeid snails. The exploration of husbandry practices points to a low perception of *F. hepatica* and its impact among farmers and to risky management strategies. Further studies should be devoted to providing in-depth characterization of the occurrence of fasciolosis in Corsica and to identification of the different hotspots for transmission throughout Corsica.

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Declaration of Competing Interest

Authors declare that they have no conflict of interest.

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