Cross-transmission of resistant gastrointestinal nematodes between wildlife and transhumant sheep

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ABSTRACT

Wild and domestic ungulates can be infected with the same species of gastrointestinal parasitic nematodes. These parasites have a free leaving stage in the environment that contributes to the ease of transmission among different host species. In addition, gastrointestinal nematodes have developed resistance to anthelmintics which is now considered a major problem for the livestock sector. In a context where wild and domestic ungulates share the same pastures, the maintenance and circulation of resistant gastrointestinal nematodes between species have rarely been explored.

In the European Alps, domestic sheep are driven in the high-altitude summer pastures leaving in sympatry with wild ungulates for several months. In this study we investigated the nemabiome of domestic sheep and Alpine ibex, *Capra ibex*, in three different areas of the French Alps to evaluate the parasites circulation between the two host species. The Alpine ibex is a protected mountain ungulate that is phylogenetically related to sheep and hosts nematode species common to sheep.

Using internal transcribed spacer 2 (ITS-2) nemabiome metabarcoding, we found sheep and ibex sharing similar gastrointestinal nematodes, except for a few species, such as *Marshallagia marshalli* and *Trichostrongylus axei*. This suggests that the long-term co-occurrence of sheep and ibex on mountain pastures has promoted the exchange of gastrointestinal nematodes between the two hosts. Based on the sequencing of the isotype 1 of the beta tubulin gene, associated with benzimidazole resistance, we found resistant nematodes in all sheep farms and in all ibex populations. Our results demonstrated that Ibex can host and shed resistant strains before transhumant sheep arrive on pastures, and they can act as a reservoir or refugia for resistant gastrointestinal nematodes. The relative role of ibex to the nemabiome and in particular to the maintenance and circulation of resistant strains in sheep remain to be determined.

Introduction

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67

68   Parasites represent a large proportion of animal diversity and are key components

69   of food webs (Hudson et al., 2006) and are essential determinants of the health,

70   fitness, population dynamics and community composition of their hosts (Tompkins et

71   al., 2011). The parasites of the Nematodes class infect a wide range of species

72   worldwide, including animals and plants (Blaxter and Koutsovoulos, 2015). In

73   animals, the gastrointestinal nematode parasites are of major concern for livestock

74   productivity and security as they can impact animal health implying reduced animal

75   productions and economical losses (Charlier et al., 2020; Roeber et al., 2013).

76   To limit the parasite load and its impact on livestock health, the use of

77   anthelmintics to treat livestock against gastrointestinal nematodes is a common and

78   cost effective practice (Vercruysse et al., 2018). Nonetheless, the repeated use of

79   anthelmintics has led to the selection of anthelmintic-resistant strains of

80   gastrointestinal nematodes. Within the gastrointestinal nematodes, resistances to

81   several families of anthelmintics (e.g., benzimidazole, macrocyclic lactones and

82   levamisole) has been observed, and resistance to several drugs is increasing

83   (Bordes et al., 2020; Kaplan and Vidyashankar, 2012; Rose et al., 2015; Rose Vineer

84   et al., 2020). The first case of resistance to benzimidazole was reported in 1964 in

85   lambs from central Kentucky (Drudge et al., 1964).

86   Today, resistance to benzimidazoles is widespread throughout the world (Kaplan

87   and Vidyashankar, 2012), and is particularly common on sheep farms in Europe

88   (Papadopoulos et al., 2012; Rose Vineer et al., 2020). The mechanisms of resistance

89   to benzimidazole are well known and documented (Whittaker et al., 2017). The

90   anthelmintic effect of benzimidazoles relies on the fixation of the molecule to the

91   parasite β-tubulin isotype-1, resulting in the disruption of tubulin–microtubule

92   equilibrium (Whittaker et al., 2017). In resistant nematodes, specific mutations of the

93   β-tubulin isotype-1 gene have been correlated with the resistance to benzimidazole in

94   several gastrointestinal nematode species (Charlier et al., 2022). These mutations

95   were associated with alteration of the β-tubulin isotype-1 structure, decreasing the

96   affinity of the protein with the benzimidazole and subsequently, activity of

97   benzimidazole (Whittaker et al., 2017).

98   Some generalist gastrointestinal nematodes can infect several host species

99   (Walker and Morgan, 2014), including both domestic and wild ungulates (e.g.,

100   Beaumelle et al., 2022; Cerutti et al., 2010). The transmission of gastrointestinal
101   nematodes among hosts, even if they do not simultaneously occupy the same
102   pastures, is possible thanks to their free-living infective larval stage that may ~~rely~~
103   active ~~until~~ several months in the environment (Carlsson et al., 2013; Fiel et al., 2012;
104   Walker and Morgan, 2014). Transmitted parasites can also include gastrointestinal
105   nematodes resistant to anthelmintics. For instance, benzimidazole-resistant
106   nematodes have been detected in free-living populations of roe deer living in
107   sympatry with livestock (Chintoan-Uta et al., 2014; Nagy et al., 2017). To date, the
108   role of wild ungulates in the epidemiology of resistant nematodes remains to be
109   determined, but it has been suggested that wildlife may act as a reservoir of resistant
110   nematodes for livestock (Brown et al., 2022; Chintoan-Uta et al., 2014; Francis and
111   Šlapeta, 2023; Laca Megyesi et al., 2019; Walker and Morgan, 2014). ~~Yet, we need~~
112   ~~to investigate the presence of resistant nematodes between co-grazing wild and~~
113   ~~domestic ungulates in different contexts, i.e., for different host species, in different~~
114   ~~landscape, and under different climatic conditions, to accurately evaluate the~~
115   ~~potential role of wildlife as reservoir for anthelmintic resistant gastrointestinal~~
116   ~~nematodes.~~

117   Transhumant pastoralism is a common practice in the European Alps and
118   consists of the seasonal movement of grazing livestock from lowland areas to
119   mountain meadows in summer which provide fresh pasture for domestic ungulates,
120   i.e., mainly sheep, but also cows or goats (Biber, 2010). These mountainous areas
121   are inhabited year-round by wild ungulates, particularly those living at high altitude in
122   the European Alps, like Alpine ibex (*Capra ibex*), or Northern chamois (*Rupicapra*
123   *rupicapra*). While wild ungulates tend to avoid domestic herds spatially or temporarily
124   during the summer (Acevedo et al., 2008), certain factors may contribute to the use
125   of the same pastures by both ~~wild and domestic ungulates~~.

126   Spatial segregation between wild and domestic ungulates is usually observed
127   once livestock arrive on pasture (Brivio et al., 2022; Ryser-Degiorgis et al., 2002).
128   Livestock are generally released onto the best grazing areas during the summer
129   season ~~due to their high productivity and accessibility of water for animals, and salt~~
130   ~~licks provides essential mineral nutrients in relatively nutrient-poor alpine ecosystems~~
131   (Chirichella et al., 2014; Richomme et al., 2006; Ryser-Degiorgis et al., 2002).
132   ~~However,~~ prior to the arrival ~~of livestock~~, mountain ungulates have been observed
133   using ~~preferentially~~ the same grazing areas ~~as those used by livestock on their arrival~~

134 (Brivio et al., 2022; Ryser-Degiorgis et al., 2002) ~~and ibex, for example, have been~~
135 ~~observed to return to the sheep grazing area immediately after the sheep have left~~
136 (Ryser-Degiorgis et al., 2002). The presence of wild and domestic ungulates in
137 attracting zones such as salt licks, even if not simultaneous, offers good opportunities
138 for parasites transmission, and these areas are therefore considered hotspots for
139 parasites infection (Richomme et al., 2006; Ryser-Degiorgis et al., 2002; Utaaker et
140 al., 2023).

141 Consequently, transhumant pastoralism represents a risk of pathogens
142 transmission between wild and domestic ungulates in mountain areas (Rossi et al.,
143 2019). Pathogen exchanges at the interface of wild and domestic ungulates have
144 already been well documented. The Alpine ibex has been identified as the wildlife
145 reservoir of brucellosis (*Brucella melitensis*) which was transmitted to cattle and
146 human in the Bargy massif in northern French Alps (Marchand et al., 2017). In
147 addition, sheep have been confirmed as the domestic reservoir of ~~the~~ Border
148 disease, which induced a major ~~virus~~ outbreak in Southern chamois (*Rupicapra*
149 *pyrenaica*) population the Pyrenees (Luzzago et al., 2016). The transmission of
150 gastrointestinal nematodes has already been described between wild ungulates and
151 transhumant domestic ungulates in mountainous areas (Cerutti et al., 2010; Citterio
152 et al., 2006; Khanyari et al., 2022; Zaffaroni et al., 2000). However, no study has yet
153 investigated the transmission of anthelmintic-resistant nematode in a transhumant
154 pastoral system.

155 In this study, we investigated the community of gastrointestinal nematodes
156 infecting Alpine ibex and domestic sheep (*Ovis aries*) and the prevalence of
157 resistance to benzimidazole, in three different regions of the French Alps. The Alpine
158 ibex was close to extinction at the beginning of the 19th century but the reinforcement
159 of its populations by several reintroductions in different part of the Alps has increased
160 the species' overall abundance and range (Brambilla et al., 2022). Today,the Alpine
161 ibex species is estimated at 52 000 individuals in Europe (Brambilla et al., 2020).

162 Given that the co-occurrence of sheep and ibex on the same pastures is limited to
163 the summer period and since sheep, and that ibex usually host species-specific
164 gastrointestinal nematodes (Walker and Morgan, 2014), we expect the nemabiome to
165 be highly differentiated between the two species in the three mountain areas (H1).
166 We expect sheep to host benzimidazole-resistant strains of gastrointestinal
167 nematode, in line with the general pattern observed for sheep in France

168 (Papadopoulos et al., 2012; Rose Vineer et al., 2020). With the implementation of
169 reintroduction programs in the second half of the 20th century, ibex have colonized
170 pastures traditionally grazed by sheep. We therefore expect that ibex will also host
171 benzimidazole-resistant gastrointestinal nematodes but to a lesser extent, as
172 resistance do not represent a selective advantage for nematodes in the ibex
173 environment (Hahnel et al., 2018) (H2). Because there are very few documented ibex
174 dispersal events among the 3 ibex populations ((Brambilla, 2020), R. Papet, C. Toïgo
175 and E. Vannard, personal communication), we should observe genetic differences
176 among nematodes species/community or strains (ASV) among the populations of
177 ibex due to genetic drift (H3).
178
## Materials and Methods
180
Study area
182
183 Samples of sheep and ibex feces were collected in the French Alps in 3
184 different mountain areas (Figure 1). The Belledonne mountain is located in the
185 western part of the Alps in southeast France. The Cerces and Champsaur mountains
186 are in the north and the south parts of the Ecrins National Park, respectively (Figure
187 1).
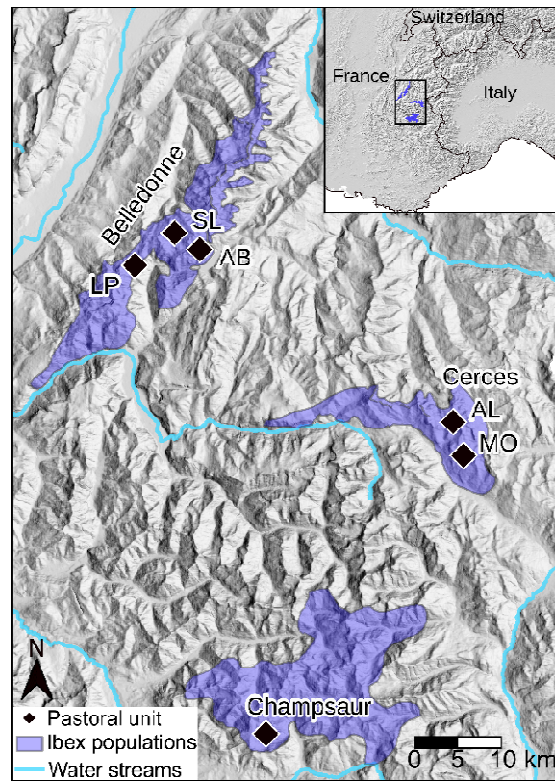
Figure 1 : Sampling locations in the French Alps ~~were~~ sheep and ibex feces were collected. Pastoral unit: area where both ibex and sheep have been sampled. Cerces : AL: Aiguillette de Lauzet, and MO: Montagne de l'Oule ; Belledonne : AB: Ane Buyant, LP : La Pesée and SL : Sept Laux.

The 3 study areas are characterized by the presence of steep slopes, high peaks (>2500m) and agropastoral activities. Climatic conditions are harsh in these mountains with a mean temperature in winter (December-March) 2015-2019 of 0°C in Belledonne (alt:1785m), 1°C in Champsaur (alt:1620m) and 1.5°C in Cerces (alt:1553m). During summer (June-September), the mean temperature is 13°C in Belledonne, 14°C in Champsaur and 16°C in the Cerces (réseau d'observation météo du massif alpin). Champsaur is the southern study area and has a Mediterranean influence. Consequently, rainfall is less important in this area compared to Cerces and Belledonne. The vegetation is distributed along an elevation gradient from coniferous woodland (*Abies alba* and *Picea abies* in Belledonne and *Larix decidua* and *Pinus sylvestris* in Cerces and Champsaur) in the lower range of ibex, to a landscape dominated by heathland with *Rhododendron ferrugineum*,

206   *Vaccinium* spp. and *Juniperus communis*, and grassland (*Carex* spp. *Festuca* spp.)

207   above the tree line (Ozenda, 1985).

208   The ibex populations were established in Belledonne, Cerces and Champsaur,

209   in 1983 with the introduction of 20 ibex, in 1959-1961 with the introduction of 6 ibex

210   and in 1994-1995 with the introduction of 30 ibex, respectively.

211   The distribution of ibex in Belledonne, Cerces and Champsaur range between

212   630m and 2860m on 200km², between 1410m and 3100m on 120km² and between

213   1320m and 3550m on 280km², respectively. Census populations sizes are estimated

214   to 800, 320 and 420 individuals in Belledonne, Cerces and Champsaur, respectively.

215   Traditional pastoral activity is practiced in all massifs, where sheep flocks

216   arrive early summer to graze mountain pastures coming from the plain on foot or by

217   truck. In Belledonne, the size of the herds are 750 ewes followed by their lambs in La

218   Pesée, 900 ewes in Sept Laux, and 1600 ewes in Ane Buyant. A dozen of rams are

219   also present within the La Pesée and Sept Laux herds, as well as some goats in La

220   Pesée. Each sheep herd from Belledonne belongs to one farmer while several

221   farmers grouped their sheep herds in Cerces and Champsaur. In Champsaur, the

222   herd included 4 breeding farms for a total of 1070 sheep and 5 goats. In Cerces, the

223   herd located in the West (Aiguillette du Lauzet) included 3 breeding farms for a total

224   of 800 sheep and the herd located in the East (Montagne de l'Oule) included 4

225   breeding farms for a total of 940 sheep.

226

227   *Sample collection*

228

229   We collected sheep feces up to 15 days after arrival on pasture to ensure that

230   we collected nematode species representative of sheep at the time of arrival on

231   pastures and to avoid detecting nematodes ingested secondarily on alpine pastures.

232   Similarly, ibex feces were collected prior to the arrival sheep and until 15 days after

233   arrival to ensure that the nematode community was not yet influenced by the arrival

234   of domestic livestock. Fresh ibex feces were mostly collected directly on the ground

235   and, in Belledonne, also during captures as part of the long-term monitoring program

236   conducted by the French Office for Biodiversity. Where possible, feces were collected

237   immediately after observation of ibex to avoid collecting of feces from the same

238   individual. Samples were stored in plastic bags, sealed after air removal, and

239 analyzed within 48h upon receipt ~~maximum 15 days after field work (mean: 2.5 [min:~~
240 ~~0 - max: 15] days)~~ in the parasitology laboratory of the National Veterinary School of
241 Lyon (ENVL, Marcy-l'Étoile, France). In total, we sampled 167 fecal samples from
242 ibex and 90 fecal samples from 6 sheep herds, distributed over four study sites, i.e.,
243 Belledonne, Champsaur, Aiguillette du Lauzet, Cerces and Montagne de l'Oule,
244 Cerces (Table 1, Figure S1).

245

246 **Table 1** : Location, period and number of samples collected in the French alps.
247 Coproscopic results of the gastro-intestinal (GI) nematodes (strongyles) eggs are
248 also reported (med[min-max]). Cerces : AL: Aiguillette de Lauzet, and MO: Montagne
249 de l'Oule ; Belledonne : AB: Ane Buyant, LP : La Pesée and SL : Sept Laux.

	Sampling date	N	GI nematodes epg
Cerces mountain			
Sheep	June 2019	AL: 15 MO: 15	AL: 7.5 [0-60] MO: 7.5 [0-225]
Ibex	AL: May-June 2019 MO: May 2018-2019	AL: 29 MO: 18	AL: 7.5 [0-30] MO: 7.5 [0-60]
Champsaur mountain			
Sheep	June 2019	15	7.5 [0-30]
Ibex	May 2019	40	7.5 [7.5-105]
Belledonne mountain			
Sheep	July 2019	AB: 15 LP: 15 SL: 15	AB: 30 [0-90] LP: 7.5 [0-165] SL: 0 [0-30]
Ibex	July 2018 and May-June 2019	80	15 [0-525]

250

251 *Parasitological analyses*

252

253 The number of gastro-intestinal nematodes eggs per gram of feces (epg) was
254 counted following a modified McMaster protocol (Raynaud et al., 1970). In order that
255 strongyles reach the L3 stage, coprocultures of feces were done at 24 ± 1 °C during
256 12-15 days with regular mixing and moistening. After the collection of L3 in tape
257 water with a Baermann apparatus, we evaluated the success of the coproculture by
258 counting the number of L3 in each sample. We extracted gastrointestinal nematodes
259 DNA from samples for which there were at least 20 L3 and we limited the extraction
260 to ~200 L3. DNA was extracted using extraction kit (Qiagen DNeasy® PowerSoil)
261 following the manufacturer's instruction with an elution volume of 50 µl of water. We

extracted twice the DNA of 30 randomly chosen samples as internal extraction control. We quantified DNA concentration for all samples using Qubit 2.0 fluorometer (Life Technologies) and ~~we~~ homogenized DNA samples to a DNA concentration of 1ng/µl (DNA samples were not diluted if the DNA concentration was <1 ng/µl).

High throughput sequencing analyses

To determine the nemabiome of sheep and ibex we used a modified version of the protocol developed by Avramenko et al., (2015). The ITS2 region of the nuclear rDNA was amplified using the primer pair NC1 (Forward - 5'-ACGTCTGGTTCAGGGTTGTT-3') and NC2 (Reverse- 5'-TTAGTTTCTTTTCCTCCGCT-3') with the following PCR conditions: 10µl of Applied Biosystems™ Master Mix AmpliTaq Gold™ 360, 5,84µl of molecular biology grade water, 0,16µl of Bovine Serum Albumin, 2µL of 5 µM mixed F and R primers form, 2 µL of DNA lysate. The PCR was performed under the following conditions: 10 min initial denaturation at 95°C; 35 cycles of denaturation (30 s at 95°C), annealing (30 s at 54°C), and extension (1 min at 72°C); a final extension at 72°C for 7 min. The thermocycling parameters were choose identically to Avramenko et al., (2015). To detect the mutations responsible of the resistance of gastrointestinal nematode to benzimidazole, we used a modified protocol of Avramenko et al., (2019). We amplified the isotype-1 β-tubulin fragment comprising the codon of position 167, 198 and 200 with two pairs of primers in two independent PCR.

The PCR conditions were: 10µl of Applied Biosystems™ Master Mix AmpliTaq Gold™ 360, 5.84 µl of molecular biology grade water, 0.16 µl of Bovine Serum Albumin, 2 µL of 5 µM mixed forward and reverse primers mix, 2 µL of DNA lysate. The PCR was performed under the following conditions: 10 min initial denaturation at 95°C; 40 cycles of denaturation (30 s at 95°C), annealing (30 s at 65°C), and extension (30 s at 72°C); a final extension at 72°C for 7 min. We targeted *Teladorsagia circumcincta* and *Trichostrongylus* spp. (Forward:5'-CGCATTCWCTTGGAGGAGG-3' and Reverse: 5'-GTGAGYTTCAAWGTGCGGAAG-3') and *Haemonchus contortus* (Forward:5'-CGCATTCYTTGGGAGGAGG-3' and Reverse: 5'-GTGAGTTTYAAGGTGCGGAAG-

295   3') with the primers described by Avramenko et al., (2019). All forward and reverse

296   primers were tagged at 5' in order that each sample had a unique combination of

297   tagged primers.

298   In all PCRs, we added positive PCR controls (i.e., *Haemonchus contortus* and

299   *Teladorsagia circumcincta* DNA extracts), negative PCR controls (distilled H$_2$O) and

300   negative DNA extraction controls. All samples (including controls) were

301   independently amplified 4 times ~~to ensure reliability of the sequencing,~~ in 96-well

302   plates ~~including~~ 209 samples, 17 PCR positive controls, 13 PCR negative controls, 7

303   extraction negative controls, 30 DNA extraction controls, as well as 12 empty wells in

304   each ~~96-well~~ plates to quantify tag jumping during PCR and sequencing steps (Figure

305   S2) (De Barba et al., 2014; Taberlet et al., 2018).

306   All PCR products of the ITS2 and the two β-tubulin isotype 1 sets were purified

307   using QIAquick® Spin Columns (QIAquick® PCR Purification KitQIAGEN) and

308   quantified using a Qubit 2.0 fluorometer (Life Technologies). Next, we pooled the 3

309   purified DNA pools (ITS2, two β-tubulin isotype 1) based on their initial concentration

310   and in proportion according to the following ratio: ITS2 50%, β-tubulin isotype 1 25%

311   for each. According to preliminary tests, we expect to achieve a sequencing depth of

312   20 000 reads per ITS2 DNA sample and 5 000 reads per β-tubulin isotype 1 DNA

313   sample. Sequencing was performed with pair-end sequencing technology on the

314   Illumina platform (2*250 bp Miseq) at Fasteris, Geneva, Switzerland.

315

316   *Sequence analysis and taxon assignation*

317

318   The sequence reads were first analyzed with the OBITOOLS package (Boyer et al.,

319   2016). Forward and reverse reads were assembled with the *alignpairedend* function,

320   and we kept only sequences with a good score of alignment (rnorm>0.8). Sequences

321   were attributed to their samples with the *ngsfilter* function with default parameters.

322   Subsequently, assigned sequences were analyzed with the dada2 package

323   (Callahan et al., 2016) following the pipeline available in www.nemabiome.ca. The

324   *dada2* pipeline returns Amplicon Sequence Variant (ASV) which are sequence

325   variants differing by as little as one nucleotide (Callahan et al., 2017). Following

326   Beaumelle et al.~~,~~ (2021), ~~the~~ gastrointestinal nematodes were identified with three

327   different methods of assignation: BLASTn (Altschul et al., 1990) against the NCBI

328 database (November 2022), and AssignTaxonomy (Callahan et al., 2016; Wang et

329 al., 2007) and IDTaxa (Murali et al., 2018) against the nematode ITS2 rDNA

330 database 1.1.0 (Workentine et al., 2020) or the nematode β-tubulin isotype 1 DNA

331 reference sequence supplied in Avramenko et al., (2019). We chose to attribute a

332 confidence level to taxonomic identification at species level: high or moderate

333 confidence if the three or two methods of assignation, respectively, were congruent.

334 We also adjusted the sequence filtering based on an adapted procedure of

335 Calderón-Sanou et al., (2020). We kept only ASVs present in at least 2 replicates of

336 the same samples and removed ASVs that were not assigned to genus level for the

337 ITS2 and species level for β-tubulin isotype 1. We removed potential contaminants

338 (reagent contaminants and cross-contaminations) following the procedure detailed in

339 Calderón-Sanou et al., (2020). For each sample, we sum the reads of the two

340 replicates with the highest similarity and if this similarity is higher than the mean

341 similarity among all replicates. At the end, we removed samples if they have <1000

342 reads of ITS2 and <500 reads of β-tubulin isotype 1 (Figure S3).

343

344 *Identification of non-synonymous mutations in codons 167, 198 and 200*

345

346 For each nematode species, all β-tubulin isotype 1 ASVs were aligned to one

347 of the β-tubulin isotype 1 consensus sequences of the reference database

348 (Avramenko et al., 2019) using the *AlignSeqs* function of the DECIPHER package

349 (Wright, 2016). We examined each β-tubulin isotype 1 ASV at codon 167,198 and

350 200 positions to record whether the codon is associated with a non-synonymous

351 mutation. However, we ignored the other polymorphism sites of the Exon.

352

353 *Statistical analyses on measures of nemabiomes*

354

355 To measure the differences of nemabiome among the two host species (sheep and

356 ibex) and the 4 study sites (Aiguillette de Lauzet, Montagne de l'Oule, Champsaur

357 and Belledonne), we considered two measures of diversity, i.e., the alpha diversity

358 and the beta diversity. The alpha diversity was measured with the Shannon index

359 that considers richness and evenness of communities and the beta diversity was

360 measured with the weighted UniFrac index estimated using the R phyloseq package

361   (McMurdie and Holmes, 2013). The weighted UniFrac distance is a phylogenetic

362   distance between the set of ASV from each nemabiome weighted by the transformed

363   abundance of each ASV (Lozupone and Knight, 2005). The phylogenetic distances

364   were computed from a phylogenetic tree which was ~~conducted~~ using ~~a~~ maximum

365   likelihood ~~tree~~ with the GTR+G+I model according to the *ModelTest* function (Posada

366   and Crandall, 1998; Schliep, 2011). The exact count ITS2 reads were transformed

367   with the Hellinger transformation (e.g., square root ~~on~~ relative frequencies) to account

368   for the high number of ~~zero in community table~~ and to decrease the influence of rare

369   ASVs in statistical analyses (Legendre and Legendre, 2012).

370   We tested the effects of host species and site, including their interaction, on alpha

371   diversity with linear models, and on beta diversity using perMANOVA (*adonis2*,

372   vegan R package (Oksanen et al., 2020)). All possible models including the null

373   model were computed. For perMANOVA models, we used a custom function to

374   compute Akaike's information criterion corrected for small sample size (AICc) based

375   on residual sums of squares (Dyson, 2018). In a model selection approach, for both

376   alpha and beta diversity, all possible models were ranked using the AICc and we

377   selected the model with the lowest AICc value. Models with ΔAICc ≤ 2 were

378   considered equivalent (Burnham and Anderson, 2002), and in this case, we

379   considered the most parsimonious one, i.e., the model with the lowest degree of

380   freedom.

381   All analyses were carried out using R 3.6 (R Core team, 2020. https://www.R-

382   project.org/).

383

384   *Statistical analyses on measures of resistant nematode strains*

385

386   To compare the importance of benzimidazole resistance ~~of~~ gastrointestinal

387   nematodes between ibex and sheep and among the 3 sites, we tested if the host

388   species, the site and the ~~gastrointestinal~~ nematode species influence the relative

389   abundance of ASVs with a resistant allele. For this purpose, we used a generalized

390   linear model with a binomial family and a model selection approach such as

391   described above.

392   We used *AlignSeqs* (Wright, 2016) to generate multi-sequence aligned β-tubulin

393   isotype 1 haplotype data. For each gastrointestinal species, we removed short ASVs,

394   e.g., ASVs with a sequence length <10% comparing to the median ASVs length.

395   PopART v1.7 (Leigh and Bryant, 2015) was used to draw the median joining

396   networks based on each gastrointestinal nematode haplotypes data.

397

398   **Results**

399

400   *Parasite material*

401

402   The median number of eggs of strongyles per gram of feces were lower in sheep

403   (7.5[0,148]$_{95\%IQR}$; n = 90) than in ibex (15[0,163]$_{95\%IQR}$; n = 167) feces (Mann–

404   Whitney U test; W = 9043.5, P = 0.006). As a result of the low level of infestation in

405   some samples, the number of L3 hatched from eggs were not sufficient (<20) for 48

406   ibex or sheep samples. These samples were not used for subsequent genetic

407   investigations. Specifically, all samples from the Sept Laux sheep herd (n=15,

408   Belledonne) were discarded. Therefore, the nemabiome was determined based on

409   the ITS2 for 196 (n= 55 sheep and n=141 ibex) out of 209 samples for which DNA

410   was extracted (Figure S1).

411

412   *Diversity of gastrointestinal nematodes in sheep and ibex*

413

414   In total, we detected 408 ASVs corresponding to 13 gastrointestinal nematode

415   species (Table 2, Figure S4). Eight ASVs were assigned to the genus level (i.e.,

416   *Marshallagia* spp., *Nematodirus* spp. and *Trichostrongylus* spp.) due to non-identical

417   assignation among taxonomic methods. An ASV corresponding to the lungworm

418   *Cystocaulus ocreatus* were discarded for the statistical analyses because we only

419   focus on gastrointestinal nematodes.

420   *Teladorsagia circumcincta* was the most prevalent nematode species and was

421   detected in 85% of samples (90%, n=127/141 ibex and 71%, n=39/56 sheep),

422   followed by *Trichostrongylus vitrinus,* 63% (73%, n=103/141 ibex and 36%, n=20/55

423   sheep) and *Haemonchus contortus,* 56% (70%, n=98/141 ibex and 22%, n=12/55

424   sheep) (Table 2, Figure S4). *Nematodirus* spp. and *Ostertagia leptospicularis* were

425   the rarest species and were detected in only 2 samples and 1 sample, respectively

426   and with a very small relative frequency (<0.1%).

427 The Nematodirus were not considered for the following results as our protocol is not

428 appropriate for this species (Hoberg et al., 2001)

429

430 **Table 2** : Number of ASV, reads and samples for each nematode taxon, including the

431 results from the ITS2 (nemabiome) and the β-tubulin isotype 1 (resistance to

432 benzimidazole). N= number ~~and percentage~~ of samples in which ~~the taxa~~ was

433 detected. Host species and study sites are mixed here.

	ITS2			β-tubulin isotype 1		
	~~ASV~~	**Reads**	**N (%)**	~~ASV~~	**Reads**	**N (%)**
Bunostomum trigonocephalum	8	61 631	12 (6%)	-	-	-
Chabertia ovina	19	95 585	62 (32%)	-	-	-
Cooperia curticei	4	803	6 (3%)	-	-	-
Cooperia fuelleborni	2	358	5 (3%)	-	-	-
Haemonchus contortus	47	1 937 587	110 (56%)	38	392 110	96 (62%)
Marshallagia marshalli	23	338 064	57 (29%)	-	-	-
Marshallagia spp.	2	404	2 (1%)	-	-	-
Oesophagostomum venulosum	13	311 530	82 (42%)	-	-	-
Ostertagia leptospicularis	1	5	1 (1%)	-	-	-
Ostertagia ostertagi	2	269	2 (1%)	-	-	-
Teladorsagia circumcincta	235	3 311 094	166 (85%)	310	343 551	145 (94%)
Trichostrongylus axei	26	462 936	103 (53%)	33	68 414	104 (68%)
Trichostrongylus colubriformis	12	411 776	102 (52%)	8	144 643	112 (73%)
Trichostrongylus vitrinus	9	521 817	123 (63%)	44	92 834	107 (69%)
Trichostrongylus spp.	5	71 124	70 (36%)	-	-	-

434

435 According to the model selection approach, ~~the~~ host species was the only

436 factor that explained the alpha diversity (Table S1). The model indicate~~s~~ that sheep

437 had a lower alpha diversity compared to ibex (β = -0.42 ± 0.07, P < 0.001, R² of the

438 model=0.15) (Figure 2b). ~~The~~ site was not retained in the ~~model selected to explain~~

439 ~~the alpha diversity~~ (Table S1).

440 Beta diversity ~~is~~ best explained by both factors, host species ($F_{1,188}$ = 27.69, P

441 = 0.001) and site ($F_{3,188}$ = 16.39, P = 0.001) and their interaction ($F_{3,188}$ = 25.61, P =

442 0.001) according to ~~our~~ model selection 💬 able 3, Table S2). Some gastrointestinal
443 nematodes were mostly ~~(*Trichostrongylus axei*: mean reads relative abundance~~
444 ~~(RRA) of 19% [11%; 26%]$_{95CI}$ in sheep feces and 2% [1%;2%]$_{95CI}$ in ibex feces) or~~
445 ~~only (*Bunostomum trigonocephalum, Cooperia* spp.)~~ found in ~~in~~ sheep feces or only
446 in ibex feces (~~*Marshallagia* spp., *Ostertagia* spp.;~~ Figure 2a). *Trichostrongylus*
447 *colubriformis* was more frequent in the Ecrins national park (mean RRA: 10% [6%;
448 13%]$_{95CI}$) than in Belledonne (mean RRA: 4% [2%; 6%]$_{95CI}$). Likewise, *Marshallagia*
449 spp. was more frequent in ibex feces in Cerces and Champsaur mountains (mean
450 RRA: 11% [6%;15%]$_{95CI}$) than in ibex feces in Belledonne (0.4% [-0.2%; 1%]$_{95CI}$). The
451 distribution of *Haemonchus contortus* in host species and sites had a particular
452 pattern. This parasite was more frequent in ibex feces compared to sheep feces in
453 Belledonne (mean RRA: 0.004% in sheep; 48% in ibex) and Champsaur (mean RRA:
454 0% in sheep; 41% in ibex), while the opposite was observed in the Cerces (Aiguillette
455 du Lauzet: mean RRA of 7% in sheep and 0.9% in ibex; Montagne de l'Oule: mean
456 RRA of 30% in sheep and 0% in ibex).

457

458 **Table 3** : Parameters estimated from the best PerMANOVA model explaining the
459 beta diversity in ibex and sheep. The effect of host species (ibex or sheep) and site
460 (Aiguillette de Lauzet, Montagne de l'Oule, Champsaur or Belledonne) and the
461 interaction between the two factors are reported. Partial R² are reported with the
462 corresponding *F*-value and *p value* (P).

Diversity index	Best model selected	Variables	partial R²	F-value	P
Weighted UniFrac	β ~ Site x Host species	Residuals	0.55	-	-
		Site	0.14	16.39	0.001
		Host species	0.08	27.69	0.001
		Site: Host species	0.22	25.61	0.001

463

464
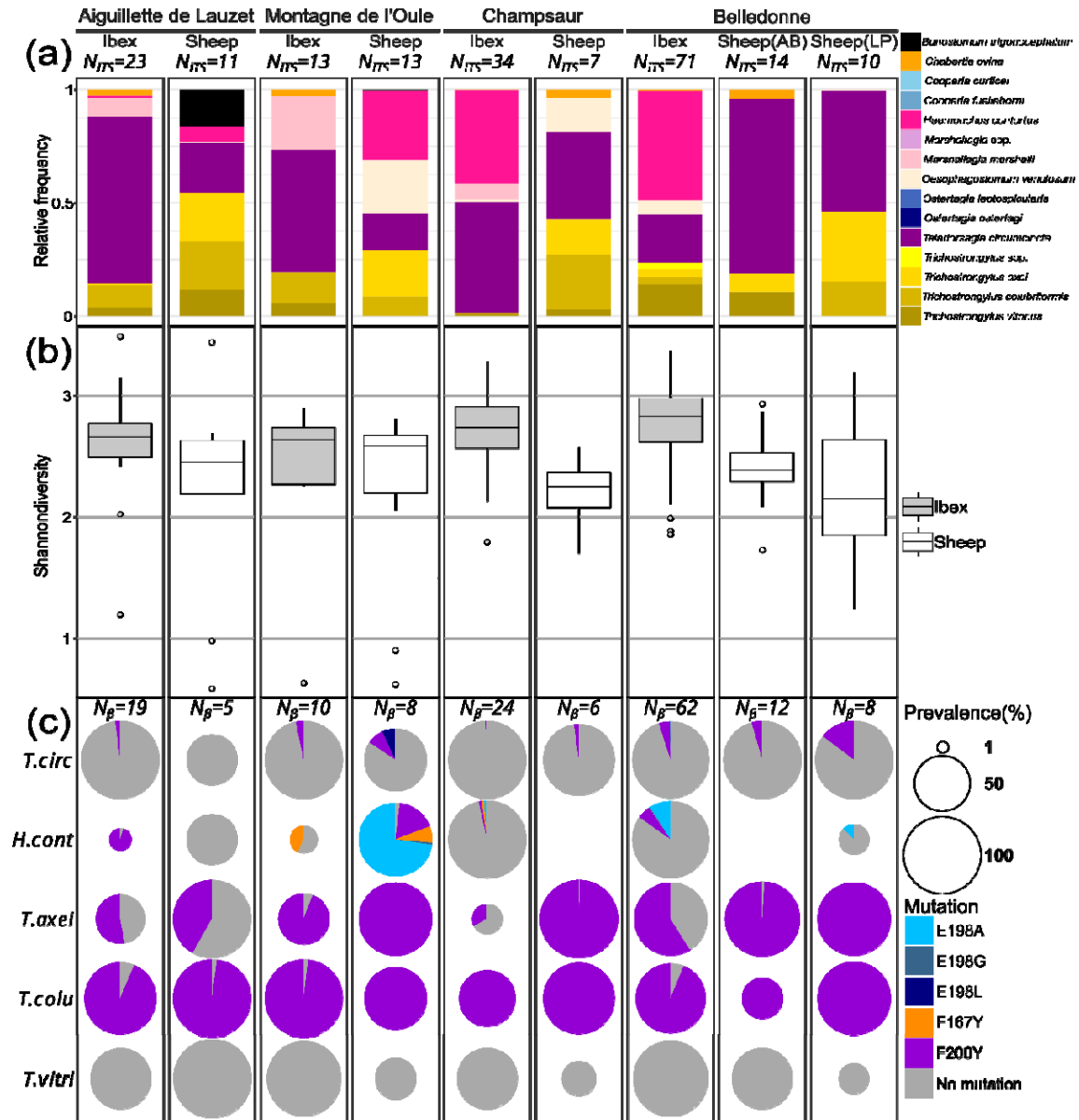
Figure 2 : (a) Mean relative frequencies of gastrointestinal nematodes species ~~and~~ (b) Shannon diversity of ITS2 ASVs and (c) prevalence and mean relative frequencies of β-tubulin isotype 1. For each host species (sheep or ibex) and each site (Cerces: Montagne de l'Oule and Aiguillette de Lauzet; Champsaur and Belledonne). The sample size for ITS2 ASVs (N_{ITS}; panel (a)) and for β-tubulin isotype 1 (N_{β}; panels (b)) is given for each population. On ~~the~~ panel (c), the size of the pie chart corresponds to the prevalence of the corresponding gastrointestinal nematode species in the population, and the size of each ~~sector~~ to the mean proportion of each allele.

Anthelmintic resistance

We found 433 different β-tubulin isotype 1 ASVs in 154 (n= 39 sheep and n=115 ibex) out of 209 samples for which DNA was extracted. Among the 5 gastrointestinal nematode species targeted by primers, we detected, *H. contortus* in 96 samples, *Teladorsagia circumcincta* in 145 samples, *Trichostrongylus axei* in 104 samples, *Trichostrongylus vitrinus* in 107 samples, or *Trichostrongylus colubriformis* in 112 samples (Table 2, Figure S5). No resistance mutation has been detected for *T. vitrinus.* Therefore, *T. vitrinus* was not included in the model explaining the relative abundance of resistant reads.

Resistance mutations were highly frequent (93.5%; n=144/154) with only 10 ibex feces (3 from Belledonne and 7 from Champsaur) in which no resistant mutation has been detected.

Based on the best model for resistant RRA, the frequency of resistant nematodes depended on gastrointestinal nematode species and the interaction between host species and the study site (Table 4, Table S3). *Teladorsagia circumcincta* was the species with the lowest resistant RRA and the nematode species with the higher resistant RRA was *Trichostrongylus colubriformis* (Table 4). The mean observed RRA of resistant nematodes differed between GIN species (*H. contortus:* 19% [13;25]$_{95CI}$; *T. circumcincta:* 4% [3;6]$_{95CI}$; *T. axei:* 70% [63;78]$_{95CI}$; *T. colubriformis:* 96% [93;99]$_{95CI}$). The resistant RRA is generally lower in ibex compared to sheep (β = -2.21 ± 0.66, *P* < 0.001). Resistant RRA were the lowest in the Aiguillette de Lauzet but this is also the only site were ibex had significantly higher resistant RRA than sheep (Table 4).

The most frequent resistance mutation is the F200Y present in the 4 gastrointestinal nematode species and 141 feces samples, followed by the E198A (46 samples, 2 nematodes species: *H. contortus* and *T. circumcincta*), the F167Y (17 samples, 2 nematodes species: *H. contortus* and *T. circumcincta*), the E198L (7 samples, *T. circumcincta*) and the E198G (4 samples, *H. contortus*) (Figure 2c).

Table 4 : Parameter estimates for the best generalized linear model explaining the resistant reads relative abundance (RRA) in ibex and sheep. The effect of host species (sheep as reference), study sites (Belledonne as reference), and their interaction, in addition to the nematode species (*Teladorsagia circumcincta* as

510 reference) are reported. Parameter estimates with standard error (SE) are reported
511 with the corresponding *z-value (z-*val*)* and *p value* (*P*). AL: Aiguillette de Lauzet; MO:
512 Montagne de l'Oule; Ch: Champsaur; Hc : *Haemonchus contortus*; Ta:
513 *Trichostrongylus axei*; Tcol: *Trichostrongylus colubriformis*.

Best model selected	Variables	Parameter estimate ± SE	z⯑val	*P*
Resistant RRA ~ host species x study sites + nematode species	Intercept	-2.02 ± 0.59	-3.45	5e-04
	Species	-2.21 ± 0.66	-3.35	8e-04
	Mountain(AL)	-2.89 ± 0.99	-2.93	0.003
	Mountain(MO)	-1.37 ± 0.89	1.54	0.123
	Mountain(Ch)	-0.10 ± 1.18	-0.08	0.935
	Nematode(Hc)	2.52 ± 0.62	4.05	5e-05
	Nematode(Ta)	4.68 ± 0.59	7.95	1e-15
	Nematode(Tcol)	7.38 ± 0.75	9.79	<2e-16
	Species:Site(AL)	2.90 ± 1.19	2.44	0.015
	Species:Site(MO)	0.04 ± 1.28	0.03	0.973
	Species:Site(Ch)	-0.75 ± 1.32	-0.56	0.574

514

515 Sheep and ibex shared 164 (38%) β-tubulin isotype 1 haplotypes and 238
516 (55%) β-tubulin isotype 1 haplotypes was only found in ibex samples (Figure 3). Most
517 of the resistant haplotypes of *T. circumcincta* and *H. contortus*, e.g., containing a
518 non-synonym mutation to the codon 167, 198 or 200, were genetic variants of a
519 common sensitive haplotype shared by ibex and sheep (Figure 3). The resistant
520 haplotypes of *T. axei* and *T. colubriformis* were more common than the sensitive
521 haplotypes and the most similar sensitive haplotypes are found either in both sheep
522 and ibex samples, or only in ibex samples. Both, *T. colubriformis* and *T. vitrinus*
523 showed two distinct lineages, separated by ≥10 mutations. One of the lineages of *T.*
524 *vitrinus* was only found in ibex from Belledonne and an ibex from Champsaur (Figure
525 3) while the ASVs of the second lineage are found both in ibex and sheep. One of the
526 lineages of the *T. circumcincta* was more diverse (Figure 3).

527
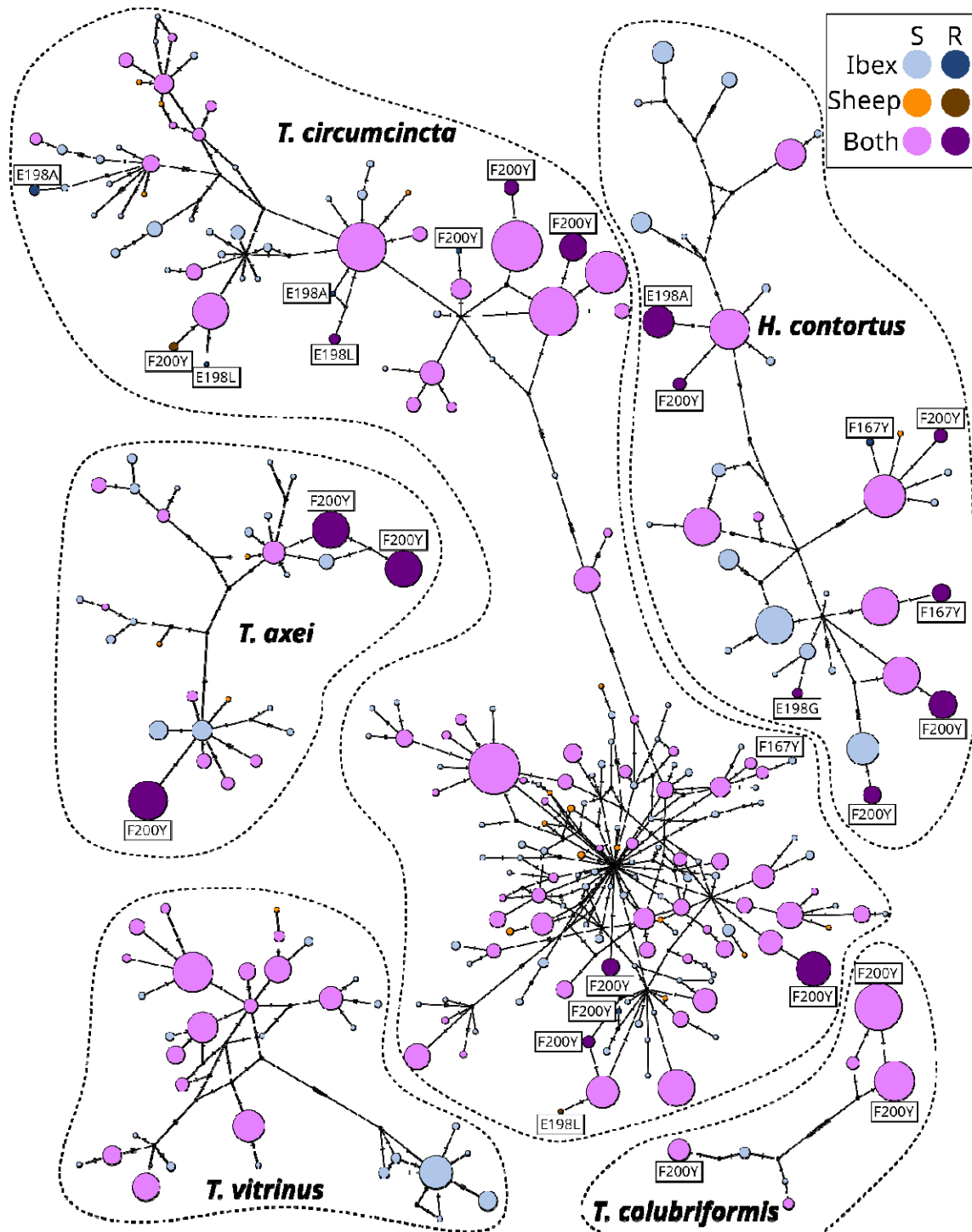
528
Figure 3 : Median joining network of β-tubulin isotype 1 haplotypes. Each point represents a unique haplotype, and the colors correspond to the host species in which the haplotype was detected. The size of the points is proportional to the number of samples in which the haplotype was found. S: sensitive haplotype, R:

533 resistant haplotype. The tag above the points indicated the name of the mutation,

534 based on the codon position and the substitution of the amino acid.

535

Discussion

537

538 Because transhumant sheep and resident Alpine ibex use the same pastures

539 during the summer, we sought to assess the extent of nematode parasites sharing

540 between these two host species. Specifically, we investigated the presence of

541 anthelmintic-resistant nematode strains in sheep and ibex to determine the role of

542 transhumant sheep in contaminating alpine pastures, and whether ibex may play a

543 role in the maintenance and circulation of anthelmintic resistant nematodes. We used

544 a metabarcoding approach based the sequencing of ITS2 and the β-tubulin to

545 demonstrate that both sheep and ibex were infected by the same gastrointestinal

546 nematode species and shared anthelmintic-resistant strains, despite the absence of

547 sheep on alpine pastures for much of the year and therefore a narrow temporal

548 window of contamination.

549 In line with other studies investigating the gastrointestinal nematodes of sheep

550 and ibex (Burgess et al., 2012; Gruner et al., 2006; Redman et al., 2019; Zaffaroni et

551 al., 2000), the most prevalent and abundant species in both host species was

552 *Teladorsagia circumcincta*. Next, *Trichostrongylus vitrinus* was moderately prevalent,

553 but not abundant in sheep and ibex nemabiome. In accordance with the climatic

554 conditions of ibex environment, these two nematode species, as well as *Marshallagia*

555 spp., are better adapted to cold temperatures than the other nematode species

556 detected in this study (O'Connor et al., 2006; Zaffaroni et al., 2000). The sheep flocks

557 originate from French plains and/or the south of France and are driven into mountain

558 areas in early summer. Consequently, their nemabiome at the time of sampling is

559 representative of the gastrointestinal nematode communities present in sheep on the

560 farm, i.e., prior to transhumance. In a similar context, Gruner et al., (2006), observed

561 a high prevalence of *T. circumcincta* in two of three transhumant sheep flocks at the

562 beginning of the grazing season in the mountains south of the Alps. Furthermore,

563 transhumant sheep flocks appear to ingest mainly *T. circumcincta* when grazing in

564 the mountains, as this parasite remains the dominant species identified in feces and

565 tracer lambs during the summer (Gruner et al., 2006). Pastoral activity in

566 mountainous areas of France could therefore favor nematode species more adapted

567 to cool and wet environmental conditions, such as *T. circumcincta* (O'Connor et al.,

568 2006), compared with sheep grazing on the plains all year round. To confirm this

569 hypothesis, a study of the variation in the nemabiome of transhumant sheep during

570 the summer should be undertaken.

571 High relative frequency (>30%) of *H. contortus* was detected in ibex in Belledonne

572 and Champsaur. In contrast, almost no *H. contortus* was observed in sheep flock

573 droved in these mountains, raising the possibility that ibex may be contributing to the

574 reinfection of sheep with *H. contortus*. To our knowledge, this is the first time that

575 such relative abundance of *H. contortus* is reported in Alpine ibex (see previous

576 studies based on morphological identification: Carcereri et al., 2021; Marreros et al.,

577 2012; Zaffaroni et al., 2000). In addition, it should be noted that Alpine ibex were

578 sampled before a potential contamination by domestic sheep could be detected, i.e.,

579 before the end of the pre-patent period: time between the infestation and the first

580 eggs production, and at the end of spring – early summer, i.e., at the start of the

581 epidemiological period at risk for *Haemonchus contortus* in a high-altitude mountain

582 area. We can therefore expect higher levels of contaminations in late summer, when

583 domestic sheep leave mountain pastures. In addition, we cannot exclude that some

584 laboratory issues might have reduced the apparent prevalence and abundance of *H.*

585 *contortus* as some samples from sheep have been kept in the fridge at 4°C during 2

586 to 3 days (including e.g., the sheep samples without *H. contortus* from Belledonne

587 and Champsaur) which can reduce the proportion of *H. contortus* eggs hatching

588 (McKenna, 1998).

589 The detection of *H. contortus* raises conservation issues for Alpine ibex as this

590 nematode species is known to be highly pathogenic to sheep (Taylor et al., 2015).

591 Infection of a phylogenetically related species, the Pyrenean ibex (*Capra pyrenaica*

592 *pyrenaica*), with a few thousand of *H. contortus* resulted in severe clinical signs,

593 including extremely low weight and hemorrhagic anemia (Lavín et al., 1997). In

594 addition, *H. contortus* may have been involved, along with pneumonia, in the collapse

595 of the Northern Chamois in the province of Lecco, Italy from November 2000 to

596 March 2001 (Citterio et al., 2006). As gastrointestinal nematodes can have an impact

597 on the demographic dynamics of the host population (Acerini et al., 2022; Albery et

598 al., 2021; Albon et al., 2002), they are suspected of being involved in the low natality

599 rates observed in the French Alpine ibex populations (Brambilla et al., 2020). While

Alpine ibex appears to be fairly resilient to parasite infections (Marreros et al., 2012), further investigations should be carried out to assess the consequences of gastrointestinal nematodes infections for ibex at both individual and population level.

We detected anthelmintic resistant alleles in 4 out of the 5 nematodes species, namely *H. contortus*, *T. circumcincta, Trichostrongylus axei, Trichostrongylus colubriformis*, but not *T. vitrinus*, for which the β-tubulin amplicons have been sequenced. Both sheep and ibex hosted resistant strains of the 4 nematode species and only 10 out of 116 ibex did not have resistant strains to any of the nematode species studied. The benzimidazole resistance was therefore very common in the studied sheep flocks, in agreement with the situation of sheep farms in Europe (Rose et al., 2015; Rose Vineer et al., 2020). The presence of anthelmintic resistant nematodes in ibex is most likely explained by the indirect transmission of resistant nematodes from sheep to ibex through the environment. The large number of shared β-tubulin ASVs between sheep and ibex and the high overlap between their nemabiome confirm this scenario (Figure 2c, Figure 3). This is in accordance with other studies investigating the share of nematode parasites at the interface of wild and domestic ungulates (Beaumelle et al., 2022; Cerutti et al., 2010; Laca Megyesi et al., 2019).

It is worth noting that feces of ibex were sampled before the arrival of sheep on pastures. This important result demonstrates that anthelmintic resistant nematodes can be maintained in mountainous areas from year to year in wild populations of ibex despite harsh winter environmental conditions, and in the absence of the main source of parasites during most of the year, i.e., the domestic sheep. The shedding of eggs from resistant nematodes by ibex prior to the arrival of domestic sheep suggests the potential role of ibex as a reservoir of anthelmintic resistant nematodes for other susceptible domestic and wild ungulates. In addition, the position of resistant mutant strains detected in ibex at the periphery of haplotypes networks (Figure 3) supported the relatively recent selection of benzimidazole resistance and the lack of benzimidazole resistant reversion since the resistant strains have been transmitted to ibex.

The 5 nematodes species for which we have studied the resistances seemed to have different selection dynamics which may reflect the life history traits of species (Redman et al., 2015). In fact, we detected no resistant allele in *T. vitrinus* and conversely, the proportion of benzimidazole resistant strains of *T. axei* and *T.*

634 *colubriformis* were high in sheep and ~~in a lesser extent~~ in ibex (Figure 2c). The
635 ~~resistance~~ proportions of *T. circumcincta* and *H. contortus* were lower compared to *T.*
636 *axei* and *T. colubriformis*, excepted for the *H. contortus* of the sheep flock ~~of the~~
637 ~~Montagne de l'Oule~~. In this study area, the proportion of resistant strains of *H.*
638 *contortus* was very high.

639 Consistent with our study, benzimidazole-resistant strains of *T. vitrinus* were rare
640 in other studies of sheep farms (in the UK, Avramenko et al., 2019, and in Canada,
641 Queiroz et al., 2020). In contrast, high frequencies of benzimidazole resistance in *T.*
642 *axei* and *T. colubriformis* (between 40% and 100%) were already reported in sheep;
643 in UK, *T. axei*: 26-27% and *T. colubriformis:* 53-62% (Avramenko et al., 2019); in
644 Austria, *T. colubriformis:* 77%-100%, (Hinney et al., 2020); in France, *T. axei*: 63%,
645 (Palcy et al., 2010). In contrast, Hinney et al., (2020), observed ~~in~~ transhumant sheep
646 flocks in Austria Alps~~,~~ a higher mean frequency of the F200Y resistance allele ~~in T.~~
647 ~~circumcincta: 32.4 ± 6.8% (mean ± standard error of the mean) and H. contortus:~~
648 ~~91.9 ± 3.7%~~, compared to the sheep flocks of this study (*T. circumcincta*, 6.6 ± 3.5%
649 and *H. contortus,* 69.9 ± 14.4%, all resistant alleles combined).

650 Several factors are suspected to contribute to interspecific differences in the
651 selection of resistance strains between nematode species, including specific
652 reproductive rate, seasonal dynamics, climatic conditions in the location of sheep
653 farms, anthelmintic strategies, e.g., treatment molecules, timing and rate of
654 anthelmintic treatments, grazing management and the cost of benzimidazole
655 resistance (Hodgkinson et al., 2019; Redman et al., 2015). However, the links
656 between ~~traits of parasites~~ and interspecific variation of resistant acquisition by
657 gastrointestinal nematodes ~~have~~ not been tested yet (Morgan et al., 2019). Our
658 results suggested a few clues in relation to the ecology of nematode species.

659 Firstly, nematode species have different abilities to practice hypobiosis, i.e., the
660 ability to halt embryonic development under environmental constraints (Gibbs, 1986).
661 *H. contortus* and *T. circumcincta* are known to arrest development more frequently
662 than *Trichostrongylus* spp. (Langrová et al., 2008), and hypobiotic larvae have been
663 shown to be less sensitive to drug (Sargison et al., 2007). Secondly, among the
664 *Trichostrongylus* spp., *T. vitrinus* may have a higher proportion of overwintering
665 larvae in pastures as this species is more resistant to cold temperature compared to
666 *T. axei* and *T. colubriformis* (O'Connor et al., 2006). As parasites on pasture are not

667 subject to selection pressure by anthelmintics, they are a source of susceptible
668 strains.

669   As the proportion of resistant strains is generally lower in ibex compared to sheep,
670 ibex may have contributed to a dilution effect of resistant strains, i.e., by hosting
671 susceptible nematodes. However, the role of ibex in the maintenance of a refugia
672 needs to be investigated by considering the relative number of susceptible strains
673 deposited by ibex on pasture compared with sheep. Furthermore, it seems that the
674 role of ibex in the maintenance of a refugia vary according to nematode species. For
675 example, ibex excrete a lower proportion of *T. axei* eggs than sheep (Figure 2a),
676 ~~while resistant strains are also dominant in ibex~~ (Figure 2c). In contrast, *T.
677 circumcincta* and *H. contortus* in ibex were more frequently susceptible and
678 genetically diverse (higher number of ITS2 and β-tubulin ASVs) compared with *T.
679 axei* and *T. colubriformis* ibex (Table 2, Figure 3). As *T. circumcincta* was dominant in
680 ibex, a refuge of susceptible *T. circumcincta* strains may ~~have been~~ maintained within
681 ibex and may contribute to limit the spread of resistance in sheep farms. ~~The results
682 concerning *H. contortus* were variable according to the massifs since ibex from the
683 two Cerces sites had a low amount of *H. contortus* compared to the two other
684 populations, i.e., Belledonne and Champsaur.~~

685 ~~The divergence between~~ massifs ~~can be~~ observed at the community and genetic
686 level among sheep flocks and ibex populations. Indeed, it was expected that some
687 differences in nemabiome composition would be observed between the massifs and
688 sheep flocks, given that sheep and ibex from different areas never meet (R. Papet, C.
689 Toïgo, E. Vannard, Pers. communication). Furthermore, the sheep flocks come from
690 different locations and have been subjected to different anthelmintic strategies. For
691 the ibex, differences in the original population of translocated animals (Gauthier and
692 Villaret, 1990; Kessler et al., 2022) and potential founder effect – not all parasites
693 present in the source population were present in the newly established ~~sink~~
694 population - may have had a long term impact on the composition of the nemabiome.
695 For example, the distinct population of *T. vitrinus*, found mainly in ibex in Belledonne,
696 may ~~had~~ been inherited from the founding ibex population. This highlights that
697 various reintroductions of ibex in the study area can also influence the composition of
698 the parasite community. This distinct population *of T. vitrinus* was absent in sheep
699 grazing in Belledonne, which hosted other strains of *T. vitrinus* and despite several
700 summers of co-occurrence of sheep and ibex in Belledonne. ~~In~~ is possible that sheep

701 never graze on pastures contaminated by these strains, but this observation may
702 also be due to a sampling bias as the number of sheep sampled remains low. Within
703 the same mountain area, few differences have, however, been observed among the
704 nemabiome of ibex, e.g., between the ibex of the Aiguillette de Lauzet and those of
705 the Montagne de l'Oule, whereas the sheep flocks host distinct nemabiome
706 communities.

707   In conclusion, transmissions of gastrointestinal nematodes species, including
708 resistant nematodes strains, have occurred between sheep and ibex even though the
709 contact between the two species is limited to the summer period. In this study, we
710 demonstrated more specifically that ibex can maintain and shed eggs of resistant
711 gastrointestinal nematodes despite the absence of sheep on pastures for several
712 months, suggesting a potential role of ibex as a reservoir for these nematodes.
713 However, the extent to which each host species can influence the nematode
714 community of the other during the transhumant period remains to be determined. To
715 this end, it would be useful to analyze the nemabiome of sheep and ibex before and
716 after the transhumant period. The lower proportion of resistance alleles in ibex
717 nemabiome compared with that of sheep underlines the possibility that ibex could
718 contribute to the maintenance and circulation of susceptible strains in sheep. Based
719 on our results, it seems that ibex have helped to limit the spread of anthelmintic
720 resistance of *T. circumcincta* and *H. contortus* in sheep flocks, by maintaining
721 parasite refugia not exposed to anthelmintic pressure. As with sheep and roe deer
722 (Beaumelle et al., 2022), domestic sheep contributes to the modification of the
723 nemabiome of ibex. This raises concerns about the conservation of the ibex, and the
724 consequences of strongyles infection in ibex should be investigated. Indeed, ibex is
725 characterized by low genetic diversity due to the strong demographic decline of this
726 species, followed by multiple re-introduction (Grossen et al., 2018) and a high genetic
727 structure of immunity-related loci among populations (Kessler et al., 2022) whereas
728 both neutral and adaptive genetic diversity are known to have an influence parasite
729 resistance in ungulates (Portanier et al., 2019).

730

731   **Appendices**

732

733 Supplementary data to this article can be found online in MendeleyData (DOI:
734 10.17632/cm97cg87d6.1).

Acknowledgements

736

752

Conflict of interest disclosure

754

755 The authors declare that they have no conflict of interest.

756

Data, scripts, and supplementary information availability

758

759 The bioinformatic pipeline, the ASV analysed during the current study and the R
760 script of statistic analyses are available in MendeleyData (DOI:
761 10.17632/cm97cg87d6.1).

762

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