Review by anonymous reviewer 1, 07 Nov 2023 18:38

This is a well-conceived study and has been conducted using appropriate methods. The conclusion is sufficiently supported by the data.

We thank the reviewer for her/his overall positive evaluation of our work.

1. I query the use of diversity metrics since none of the hypotheses concern diversity per se, rather the extent of overlap in parasite species and resistance genotypes between hosts. But this is a minor point and could be addressed by including an a priori hypothesis on diversity (this could be two-tailed to avoid retro-fitting expectation to data).
   We recognize that we probably didn't go into enough detail about our hypotheses concerning the diversity of parasites we might find in sheep or ibex, so we have now added at **L161-169**: "Ibex usually host species-specific gastrointestinal nematodes (Walker and Morgan, 2014) but they may also be exposed to generalist nematodes deposited by other related ungulates species, that live in the same area at least part of the year, being wild (i.e., Northern chamois, *Rupicapra rupicapra*) or domestic (e.g., sheep). Furthermore, anthelmintic treatments are frequently applied to livestock by farmers with the aim of reducing the parasite load and hence reducing the diversity of nematodes in sheep. Therefore, we expected the nemabiome to be highly differentiated between the two species in the three mountain areas, with a higher nematode diversity in ibex compared to sheep (H1)."

2. The introduction is well-written and comprehensive; could perhaps be abbreviated a little (e.g. the section on BZ-resistance history and mechanisms is not too necessary here). Methods, results and discussion are clear.
   Accordingly, we shorten the introduction by removing sentences in the section on BZ-resistance **L91-97**: "In particular, resistance to benzimidazoles is widespread throughout the world (Kaplan and Vidyashankar, 2012), and is particularly common on sheep farms in Europe (Papadopoulos et al., 2012; Rose Vineer et al., 2020). Contrary to other anthelmintic families, the mechanisms of resistance to benzimidazole are well known and documented (Whittaker et al., 2017). In resistant nematodes, specific mutations of the β-tubulin isotype-1 gene have been correlated with the resistance to benzimidazole in several gastrointestinal nematode species (Charlier et al., 2022)."

3. With regards to the likely spill-over and maintenance of resistant worms in ibex, originating from sheep, could it not also be possible that anthelmintic residues in the environment might lead to exposure of ibex worms and selection in situ?
   This is an interesting point. And indeed, exposition of nematodes to anthelmintic residues present in the environment may select in situ for resistance in nematode communities, as it has recently been suggested (Dimunová et al., 2022). In addition, sheep are generally treated just before their ascent to the mountain pastures with potential excretion of anthelmintic via feces on pastures several days or weeks after the drug administration (Kolar et al., 2006). Furthermore, active compounds may persist in the environment for weeks or months (e.g. for moxidectin: https://www.ema.europa.eu/en/medicines/veterinary/referrals/moxidectin-containing-veterinary-medicines-used-cattle-sheep-and-horses). Unfortunately, we have no idea of the amount of anthelmintics residues circulating in area used by ibex yet, but we aim at investigating this question in a near future.
   We added a section in the discussion on this perspective **L706-714**: "Whereas sheep are generally treated just before their ascent to the mountain pastures, excretion of anthelmintic via sheep feces can occur during several days after the drug administration and the molecules degradation last days, or even months (Kolar et al., 2006). In addition, sub-lethal exposition of

nematode to anthelmintic residues present in the environment may select in situ for anthelmintic resistance (Dimunová et al., 2022). Unfortunately, the level of drugs in the environment, their persistence and their spread in grazed mountainous area are totally unknown. Environmental circulation of anthelmintic residues should be investigated in further studies to understand its incidence on the presence of resistant nematodes in wildlife."

Kolar, L., Flajs, V.C., Kužner, J., Marc, I., Pogačnik, M., Bidovec, A., van Gestel, C.A.M., Eržen, N.K., 2006. Time profile of abamectin and doramectin excretion and degradation in sheep faeces. Environ. Pollut., Soil and Sediment Remediation (SSR) 144, 197–202. https://doi.org/10.1016/j.envpol.2005.12.019

Dimunová, D., Matoušková, P., Navrátilová, M., Nguyen, L.T., Ambrož, M., Vokřál, I., Szotáková, B., Skálová, L., 2022. Environmental circulation of the anthelmintic drug albendazole affects expression and activity of resistance-related genes in the parasitic nematode Haemonchus contortus. Sci. Total Environ. 822, 153527. https://doi.org/10.1016/j.scitotenv.2022.153527

4. Overall the study fails to conclude on the extent to which these worm populations are shared between hosts and the role of ibex in maintaining them and influencing genetic composition. This is sensible and justified and the authors are right to be cautious. All the same they might suggest more concretely what further studies are needed to answer this question – especially what is possible regarding longitudinal and intervention studies.

Indeed, we have been cautious in interpreting our results so as not to over-interpret the available data. To further explore the dynamic and extent to which nematodes are shared between the host populations, we need to better understand how and when the exchange occurred. To do so, a temporal longitudinal sampling, before, during and after the different species of hosts shared the same pasture should be considered. This should be coupled with the analysis of population structure with appropriate genetic markers (microsatellites or SNPs) to properly quantify gene flow among ibex and sheep nematodes populations. Alternatively, intervention studies are often required to infer the role of ibex in maintaining nematodes populations shared between the two host species (Viana et al., 2014). To refine our knowledge on the ability of ibex to maintain nematodes from sheep, and more specifically, resistant nematodes, we could either experimentally infect enclosed ibex (e.g., in zoological parks) with nematodes (see e.g. Laca Megyesi et al 2020) or restrict the accessibility of an area used by ibex to sheep and goats, and monitor the dynamic of infection of ibex by nematodes during the following weeks – months - years. The use of mathematical models could help to understand the relative contribution of domestic and wild ungulates to the dynamic of resistant nematodes (Brown et al. 2022; Dickinson et al. 2024).

We have now modified our conclusion, by adding at **L820-832**: "To this end, a temporal sampling, before, during and after the different host species share the same pasture should be considered. Analysis of parasite population structure using appropriate genetic markers (i.e., microsatellites or SNPs) should help to properly quantify gene flow between ibex and sheep nematode populations (Cerutti et al., 2010). In addition, intervention studies are required to infer the role of ibex in maintaining nematodes populations shared between the two host species (Viana et al., 2014). Experimental infections of captive ibex or monitoring free-ranging ibex populations after access to alpine pastures has been restricted to livestock should help us to

refine the ability of ibex to maintain nematodes from domestic ungulates, including resistant nematodes. Finally, epidemiological models could be useful tools to better understand the dynamics of resistant parasites at the livestock-wildlife interface (Brown et al. 2022; Dickinson et al. 2024)."

Brown, T.L., Airs, P.M., Porter, S., Caplat, P., and Morgan, E.R. 2022. Understanding the role of wild ruminants in anthelmintic resistance in livestock. Biol. Lett. 18(5): 20220057. doi:10.1098/rsbl.2022.0057.

Dickinson, E.R., McFarland, C., Toïgo, C., Michael Scantlebury, D., Stephens, P.A., Marks, N.J., and Morgan, E.R. 2024. Host movement dominates the predicted effects of climate change on parasite transmission between wild and domestic mountain ungulates. R. Soc. Open Sci. 11(1): 230469. Royal Society. doi:10.1098/rsos.230469.

Laca Megyesi, Š., Königová, A., Babják, M., Molnár, L., Rajský, M., Szestáková, E., Major, P., Soroka, J., Urda Dolinská, M., Komáromyová, M., Várady, M., 2020. Wild ruminants as a potential risk factor for transmission of drug resistance in the abomasal nematode Haemonchus contortus. Eur. J. Wildl. Res. 66, 9. https://doi.org/10.1007/s10344-019-1351-x

Viana, M., Mancy, R., Biek, R., Cleaveland, S., Cross, P.C., Lloyd-Smith, J.O., Haydon, D.T., 2014. Assembling evidence for identifying reservoirs of infection. Trends Ecol. Evol. 29, 270–279. https://doi.org/10.1016/j.tree.2014.03.002

5. The English is generally excellent but could be improved slightly in places, e.g. typos / spelling lines 44, 138, 146, 153, 163, (non-exhaustive list).
We integrated the correction of the recommender and carefully read the manuscript. The corrections are highlighted in yellow.

Review by anonymous reviewer 2, 16 Oct 2023 15:36

In my opinion, the work is original and the manuscript is well written. The authors question the interface between domestic and wild ungulates and its consequences in terms of parasite transmission in mountain ecosystems, with issues for both pastoral activities and wildlife conservation. Overall, the description of the methods and the results are clear, with sufficient details and useful illustrations; the discussion is interesting and the conclusions are adequately supported by the results. I have only minor comments and some suggestions mainly to enrich the discussion.
We thank the reviewer for her/his overall positive evaluation of our work.

6. Line 57: the results show that Ibex populations harbor resistant strains before the arrival of the sheep, showing that these strains were maintained for a year. However, there is no demonstration that this maintenance can last more than a year in the absence of sheep. The possible contribution of other wild ungulates that potentially frequent the pastures (as chamois) to the maintenance of the resistant strains has not be studied in the present works. Also, and given that the term reservoir refers to a capacity to maintain a pathogen over the long term without external input, I suggest to be more careful in the use of terms. My

suggestion would be to reformulate with "and then could act as refuge or even contribute to maintain resistant GIN". This comment also lead me to a question: is there any data, even in livestock, on the maintenance of the resistant strains in absence of any selection pressure (i.e. absence of the use of anthelminthics)? In my point of view, it would be interesting to discuss this point.

We totally agree with this comment and replace the sentence in the abstract L56-57. To our knowledge, there is no empirical studies which prove a decline of resistant strains in the absence of selection pressure in wild ungulates. At this time, the reversion of anthelmintic resistance has been demonstrated in sheep flocks by using molecules to which resistant nematodes were still susceptible (Leathwick et al., 2015) or less efficiently, by introducing susceptible strains among the resistant strains (George et al., 2021; Moussavou-Boussougou et al., 2007). We discussed this point **L722-726**: "Once resistant strains have been selected, the absence of selection pressure (i.e. absence of the use of anthelmintics) do not guarantee the reversion of resistance (Hamilton et al., 2022; Leathwick et al., 2015). Consequently, ibex could probably maintain benzimidazole-resistant strains for several years even in the absence of selection pressure.".

George, M.M., Vatta, A.F., Howell, S.B., Storey, B.E., McCoy, C.J., Wolstenholme, A.J., Redman, E.M., Gilleard, J.S., Kaplan, R.M., 2021. Evaluation of changes in drug susceptibility and population genetic structure in *Haemonchus contortus* following worm replacement as a means to reverse the impact of multiple-anthelmintic resistance on a sheep farm. Int. J. Parasitol. Drugs Drug Resist. 15, 134–143. https://doi.org/10.1016/j.ijpddr.2021.02.004

Hamilton, K.M., Waghorn, T.S., de Waal, T., Keane, O.M., Green, P., Leathwick, D.M., 2022. *In vitro* evaluation of fitness parameters for isolates of *Teladorsagia circumcincta* resistant and susceptible to multiple anthelmintic classes. Vet. Parasitol. 310, 109791. https://doi.org/10.1016/j.vetpar.2022.109791

Leathwick, D.M., Ganesh, S., Waghorn, T.S., 2015. Evidence for reversion towards anthelmintic susceptibility in *Teladorsagia circumcincta* in response to resistance management programmes. Int. J. Parasitol. Drugs Drug Resist. 5, 9–15. https://doi.org/10.1016/j.ijpddr.2015.01.001

Moussavou-Boussougou, M.-N., Silvestre, A., Cortet, J., Sauve, C., Cabaret, J., 2007. Substitution of benzimidazole-resistant nematodes for susceptible nematodes in grazing lambs. Parasitology 134, 553–560. https://doi.org/10.1017/S0031182006001697

7. Line 81: the authors introduce the fact that resistances to several families of anthelmintics have been observed but their works focus only on benzimidazole resistance. It would be interesting to explain, in the introduction or the discussion, whether similar studies but for other anthelmintics would be possible and if not to explain why.

Contrary to other anthelmintic families, the genetic mechanism of resistance to benzimidazole is well known and large scale screening of this resistance based on molecular tools is now feasible (Avramenko et al., 2019). Nowadays, the analysis of other anthelmintics needs technics which are difficult to achieve for wildlife in remote field. We completed the paragraph **L93-102** with the additional information: "Contrary to other anthelmintic families, the genetic mechanisms of resistance to benzimidazole are well known and documented (Whittaker et al., 2017). In resistant nematodes, specific mutations of the β-tubulin isotype-1 gene have been

correlated with the resistance to benzimidazole in several gastrointestinal nematode species (Charlier et al., 2022). Furthermore, large-scale screening based on molecular tools is now feasible for this resistance (Avramenko et al., 2019), whereas the recommended method in livestock (i.e., the fecal egg count reduction test; Kaplan et al., 2023) for the diagnosis of resistance to other anthelmintics requires techniques that are difficult to achieve in wildlife in remote fields."

Avramenko, R.W., Redman, E.M., Melville, L., Bartley, Y., Wit, J., Queiroz, C., Bartley, D.J., Gilleard, J.S., 2019. Deep amplicon sequencing as a powerful new tool to screen for sequence polymorphisms associated with anthelmintic resistance in parasitic nematode populations. Int. J. Parasitol. 49, 13–26. https://doi.org/10.1016/j.ijpara.2018.10.005

Charlier, J., Bartley, D.J., Sotiraki, S., Martinez-Valladares, M., Claerebout, E., von Samson-Himmelstjerna, G., Thamsborg, S.M., Hoste, H., Morgan, E.R., Rinaldi, L., 2022. Chapter Three - Anthelmintic resistance in ruminants: challenges and solutions, in: Rollinson, D., Stothard, R. (Eds.), Advances in Parasitology. Academic Press, pp. 171–227. https://doi.org/10.1016/bs.apar.2021.12.002

Kaplan, R. M., Denwood, M. J., Nielsen, M. K., Thamsborg, S. M., Torgerson, P. R., Gilleard, J. S., Dobson, R. J., Vercruysse, J., & Levecke, B. (2023). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. Veterinary Parasitology, 318, 109936. https://doi.org/10.1016/j.vetpar.2023.109936

Whittaker, J.H., Carlson, S.A., Jones, D.E., Brewer, M.T., 2017. Molecular mechanisms for anthelmintic resistance in strongyle nematode parasites of veterinary importance. J. Vet. Pharmacol. Ther. 40, 105–115. https://doi.org/10.1111/jvp.12330

8. Line 176: the acronym ASV is used here for the first time in the manuscript, please explain it.
   Done, **L179-180**: "Because there are very few documented ibex dispersal events among the 3 ibex populations ((Brambilla, 2020), R. Papet, C. Toïgo and E. Vannard, personal communication), we predicted genetic differences among nematodes species/community or strains (ASV: Amplicon sequence variant) among the populations of ibex due to genetic drift (H3)."

9. Line 194 and following: to better understand the extent of the interfaces which are studied and in what proportion these interfaces are explored in the works, it would be useful to provide elements on the extent area of the massifs and that of the sampling locations where feces were collected (if data available).
   We do not have accurate maps which could show the pastoral units and the areas where feces were collected, but we now precise in the manuscript, **L242-243** that "Ibex feces were collected within each of the pastoral units in which we collected sheep feces".

10. In this Material and method section, I suggest gathering all the descriptive data on study sites in a table to make their vizualisation easier and to quickly identify differences between sites.
    The editor also suggested that the paragraph about the study area be made easier to read. We rephrased this paragraph (**L212-228**) and added a summary table in this section.

11. Line 214: please specify whether the term “individuals” concerns all age groups or only adults.
    We now precise, **L244-245**, that “Feces from all age groups were collected”.

12. Line 219 and 222: the authors mention the presence of a goats on some pastures, but there is no further mention of goats afterwards. Since Ibex is phylogenetically closer to goat than to sheep, and that the parasitism of goats can differ from that of sheep, qualitatively and quantitatively, I would have found interesting to also explore the parasitism of the latters. I imagine that this was not done for understandable practical reasons, but I suggest that the authors address this point in the discussion (possible impact of the presence of goats on the nemabiome of Ibex?) in a paragraph that I suggest to add to discuss the possible role of other species (see my last comment).
    Indeed, several nematode species are common to several ungulate species present in the study areas (Mediterranean mouflon, *Ovis gmelini musimon* × *Ovis sp*.; Northern chamois; domestic goat, *Capra hircus*; red deer, *Cervus elaphus*; and roe deer, *Capreolus capreolus*) (Zaffaroni et al., 2000). In our study, only a few domestic goats are present in Belledonne (n = 11 individuals) and in Champsaur (n =5 individuals) and represent less than 0.01% of the domestic flock in the area. As we collected feces directly on the ground, we cannot exclude that goat feces had been collected instead of sheep feces. In our opinion, domestic goats should not have a significative influence on nemabiome of ibex in our study area considering the scarcity of the species among the sheep.

    In further analyses, we should also consider other wild ungulates leaving in the same study area. Especially since they have different space use and should provide key information to better understand the dynamic of nematodes exchanges among domestic and wild ungulates. We add in the discussion **L666-679** “Several nematode species are common to several ungulate species present in the study areas (Mediterranean mouflon, *Ovis gmelini musimon* × *Ovis sp*.; Northen chamois; domestic goat, *Capra hircus*; red deer, *Cervus elaphus*; and roe deer, *Capreolus capreolus*) (Zaffaroni et al., 2000). In our study, only a few domestic goats are present in Belledonne (n = 11 individuals) and in Champsaur (n =5 individuals) and represent less than 0.01% of the domestic flock in the area. As we collected feces directly on the ground, we cannot exclude that goat feces had been collected instead of sheep feces. In our opinion, domestic goats should not have a significative influence on nemabiome of ibex in our study area considering the scarcity of the species among the sheep. In further analyses, we should consider the different domestic and wild ungulates species leaving in the same study area. Especially because they have different space use, different nemabiome and should provide key information to better understand the dynamic of nematodes exchanges among domestic and wild ungulates.”

    Zaffaroni, E., Teresa Manfredi, M., Citterio, C., Sala, M., Piccolo, G., Lanfranchi, P., 2000. Host specificity of abomasal nematodes in free ranging alpine ruminants. Vet. Parasitol. 90, 221–230. https://doi.org/10.1016/S0304-4017(00)00240-5

13. Line 234: the authors explain that, when possible, they collected Ibex feces just after their deposit and in certain cases also during capture. In one of the files provided in appendix (Sample_description_Sheep_ivbex_all.csv), we can see that the data on the sex was registered during capture. Would it be possible to test a possible influence of sex on the results? As the use of pastures by males and females is not similar in space and time for Ibex, one hypothesis could be that the nemabiome are different. In the same way, for feces collected shortly after their emission, would it have been possible to note whether these feces was emitted by groups

of males or females to test whether it affects the diversity of gastrointestinal nematode and the anthelmintic resistance?

As it has already been shown in roe deer in Beaumelle et al. (2021), GIN community may change according to the sex and the age of the individuals. We now compared alpha and beta diversity between females and kids (N=33) and males (N=32) for sites for which we get information on the sex and age of individuals (i.e., Aiguillette de Lauzet (AL), Belledonne and Champsaur). Because site has a significant effect on GIN community and sample size are heterogenous depending on sites (Aiguillette de Lauzet: N_{males}=18, $N_{females\ and\ kids}$= 5; Belledonne: N_{males}=5, $N_{females\ and\ kids}$= 11; Champsaur: N_{males}=9, $N_{females\ and\ kids}$= 17), we used generalized linear models for alpha and perMANOVA for beta diversity, and a model selection approach to investigate the influence of sites and ibex classes ("females and kids" or "males") on GIN community.

The model selection approach retained the effects of site and class of individuals and the interaction between the two independent factors in the best model explaining alpha diversity (Table 1). We found significant differences among sites : Shannon index of alpha diversity was higher in Belledonne ($\beta = 0.62 \pm 0.16$, $P < 0.001$) and Champsaur ($\beta = 0.55 \pm 0.16$, $P < 0.001$) compared to the Aiguillette du Lauzet. The diversity of nematodes was higher also in males compared to females/yearlings ($\beta = 0.60 \pm 0.15$, $P < 0.001$), except in Champsaur where males present a lower alpha diversity than females with kids ($\beta = -0.52 \pm 0.20$, $P < 0.001$).

For the beta diversity, the best model ($\Delta AICc \leq 2$ and lowest degree of freedom) included only the effect of site ($F_{2,62} = 15.93$, $P = 0.001$, Table 2).

Table 1. Set of generalized linear models explaining the alpha diversity sorted by AICc value. The best model is highlighted in bold (i.e., the most parsimonious model among those with ΔAICc≤2).

Generalized linear models	df	AICc	ΔAICc	weight
$\boldsymbol{\alpha \sim site + classes + site \times classes}$	**7**	**40.24**	**0**	**0.72**
$\boldsymbol{\alpha \sim site + classes}$	5	42.41	2.16	0.25
$\boldsymbol{\alpha \sim classes}$	3	48.04	7.80	0.01
$\boldsymbol{\alpha \sim 1}$	2	49.16	8.92	0.01
$\boldsymbol{\alpha \sim site}$	4	49.39	9.15	0.01

Table 2. Set of perMANOVA models explaining the beta diversity sorted by AICc value. The best model is highlighted in bold (i.e., the most parsimonious model among those with ΔAICc≤2).

perMANOVA models	k	AICc	ΔAICc	weight
$\boldsymbol{\beta \sim site}$	**3**	**-187.61**	**0**	**0.45**
$\boldsymbol{\beta \sim site + classes}$	4	-187.39	0.22	0.40
$\boldsymbol{\beta \sim site + classes + site \times classes}$	6	-185.47	2.14	0.15
$\boldsymbol{\beta \sim classes}$	2	-168.26	19.35	0
$\boldsymbol{\beta \sim 1}$	1	79.39	267.00	0

For the benzimidazole resistance frequencies, the sample size is smaller: sites (AL: N_{males}=16, $N_{females\ and\ kids}$= 3; Belledonne: N_{males}=4 $N_{females\ and\ kids}$= 10; Champsaur: N_{males}=8, $N_{females\ and\ kids}$= 10). Consequently, we simplified the model and test the frequency of resistant strains from all GIN species. Based on a one-way ANOVA the frequency of resistance is not different between males and females with kids ($F_{1,49}$=0.03, P = 0.863).

We now included both tables in appendix.

We indicated in the methods L411-414: "As sex and age have been determined for some ibex, we also tested the effect of ibex classes (1: adult males, 2: females or kids/yearlings) on alpha and beta diversity following the same model selection approach, including ibex classes and sites as explanatory variables.".

We reported those results **L506-515** "The model selection approach retained the effects of site and class of individuals and the interaction between the two independent factors in the best model explaining alpha diversity (Table S3). We found significant differences among sites : Shannon index of alpha diversity was higher in Belledonne ($\beta = 0.62 \pm 0.16$, $P < 0.001$, R^2 of the model=0.27) and Champsaur ($\beta = 0.55 \pm 0.16$, $P < 0.001$) compared to the Aiguillette du Lauzet. The diversity of nematodes was higher also in males compared to females/yearlings ($\beta = 0.60 \pm 0.15$, $P < 0.001$), except in Champsaur where males present a lower alpha diversity than females with kids ($\beta = -0.52 \pm 0.20$, $P < 0.001$). For the beta diversity, the best model included only the site (($F_{2,62}$ = 15.93, P = 0.001, Table S4)." And **L555-557**, "We found no significative effect of ibex classes (males or females and kids/yearlings) on benzimidazole resistance frequencies ($F_{1,49}$=0.03, P = 0.863, ANOVA test)."

In addition, we added in the discussion **L680-692**: "Contrary to results obtained on roe deer *Capreolus capreolus* (Beaumelle et al. 2021), we found higher diversity of nematodes in adult males compared to females and kids/yearlings. In fact, ibex have high sexual dimorphisms and male are certainly more susceptible to parasitism (Markle and Fish, 2014). In addition, ibex segregate by sex (Brambilla et al., 2022), providing less opportunities for intersexual transmission of parasites. Contrary to females and kids, before the grazing period males feed on patches grazed by domestic sheep (Margaillan 2021), increasing the probability of infection of males by over-wintering nematodes deposited by livestock during the previous transhumance (O'Connor et al., 2006). On another side, we did not notice any difference between classes concerning benzimidazole resistance frequencies. However , more information regarding the spatial distribution of both sexes are required if we want to investigate further the susceptibility of one group (male or female with kids) to spread and exchange parasites with domestic livestock (Bourgoin et al., 2021)."

Beaumelle, C., Redman, E.M., de Rijke, J., Wit, J., Benabed, S., Debias, F., Duhayer, J., Pardonnet, S., Poirel, M.-T., Capron, G., Chabot, S., Rey, B., Yannic, G., Gilleard, J.S., Bourgoin, G., 2021. Metabarcoding in two isolated populations of wild roe deer (*Capreolus capreolus*) reveals variation in gastrointestinal nematode community composition between regions and among age classes. Parasit. Vectors 14, 594. https://doi.org/10.1186/s13071-021-05087-5

Bourgoin, G., Portanier, E., Poirel, M.-T., Itty, C., Duhayer, J., Benabed, S., Cockenpot, A., Callait-Cardinal, M.-P., Garel, M., 2021. Reproductive females and young mouflon (*Ovis gmelini musimon* × *Ovis* sp.) in poor body condition are the main spreaders of gastrointestinal parasites. Parasitology 148, 809–818. https://doi.org/10.1017/S0031182021000329

Brambilla, A., Bassano, B., Biebach, I., Bollmann, K., Keller, L., Toïgo, C., von Hardenberg, A., 2022. Alpine Ibex *Capra ibex* Linnaeus, 1758, in: Corlatti, L., Zachos, F.E. (Eds.), Terrestrial Cetartiodactyla, Handbook of the Mammals of Europe. Springer International Publishing, Cham, pp. 383–408. https://doi.org/10.1007/978-3-030-24475-0_32

Markle, J.G., Fish, E.N., 2014. SeXX matters in immunity. Trends Immunol. 35, 97–104. https://doi.org/10.1016/j.it.2013.10.006

Margaillan, L. (2021). Simultaneous GPS monitoring during summer reveals habitat selection in male Alpine ibex is shaped by resource and interference competition with sheep herds. Office Français de la Biodiversité, Master thesis, 31 p.

O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. Vet. Parasitol. 142, 1–15. https://doi.org/10.1016/j.vetpar.2006.08.035

14. Line 246/ table1: it is surprising that, in Belledonne, Ibex samples from 2018 and 2019 have been grouped together, without details on the number collected per year, any demonstration that the nemabiome composition is similar from one year to the next, and specification whether the pastoral pressure was similar from one year to the next (same flock size and composition?). This choice to group samples is all the more surprising as some feces were collected in July (in 2018) when one of the objectives of the work was to study parasitic diversity and resistance to anthelmintics before the arrival of the sheep (unless Belledonne is an exception, sheep are usually present on pastures since June). Finally, I did not understand why in the spreadsheet in Appendix there are 93 Ibex while table 1 indicates 80 Ibex samples in Belldone. This may be a misreading on my part, but I haven't seen any explanation of this difference. I have not compared data in detail for all the sites but it would be good to check the concordance of the numbers between the table and spreadsheet, or to explain why the data differ. In the same way, for Cerces MO, unless I am mistaken, the spreadsheet in appendix mention ibex samples in May 2019 but not in 2018, contrary to table 1.
In 2018, we collected ibex feces in early July because sheep were on mountain pasture exceptionally late in summer this year. In 2018 and 2019, the same sheep farms were present in the pastoral units. We included the samples from 2018 to increase statistical power at the risk to interannual variability in our models. Among the 80 samples of ibex collected in Belledonne, 21 were collected in 2018. The nemabiome (ITS2) was determined for 17 of them and the β-tubulin ASV were determined for 13 of them. We visually verified that nemabiome between ibex from 2018 were not different from those of 2019, and we decided to keep them in the analyses as we did not detect any particular pattern (Figure 1). This figure was included in Appendix.
We added **L251-254** "In Belledonne, 21 samples were collected in 2018 following the sampling strategy of 2019. We controlled that year of sampling did not result in drastic change of nemabiome in ibex (figure S6) and included those samples in analyses".

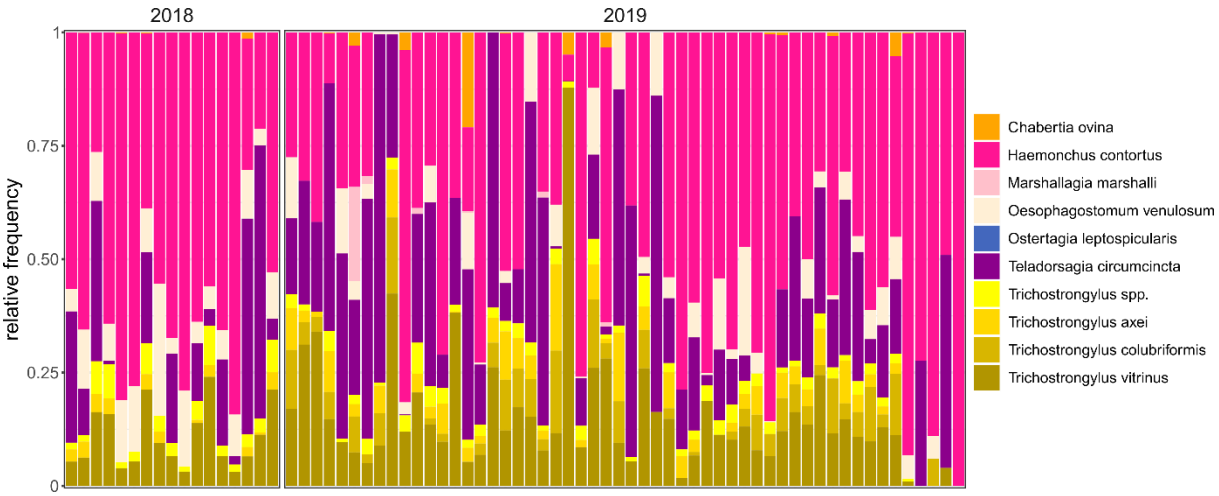

Figure 1: Nemabiome of ibex in Belledonne. Each stacked bar chart represents the species composition of ibex samples within which each taxon is defined by one color. The data are split based on year of sampling.

The 93 samples of Ibex in the spreadsheet included 13 samples from which DNA were extracted and sequenced twice. Those samples had a "R" letter added to their name. See **L288-290** in the manuscript: "We extracted twice the DNA of 30 randomly chosen samples as internal extraction controls (Taberlet et al., 2018)". We added in appendix an additional excel file to quickly resume samples used in this study. In addition, we added **L368-369** "Then, we verified that the 30 DNA extraction replicates had similar nemabiomes and kept one sample replicate out of two".

We apologize for the mistake about the year of sampling for the samples of Cerces MO in the table 2: ibex were only sampled in 2019. We corrected this mistake and ensured the concordance between the spreadsheet available in supplementary material and the manuscript.

15. Line 539: it would be useful if the authors clarified that Ibex populations use areas grazed by sheep but most on the time at different times (months of the year or times of the day).
We clarified this sentence **L600-601**: "Because resident Alpine ibex use pastures grazed by transhumant sheep during summer, but not concurrently (unpublished data)"

16. Line 543: the circulation of a pathogen being a prerequisite for its maintenance, I suggest to reverse circulation and maintenance in the sentence.
Done**. L605**; "Specifically, we investigated the presence of anthelmintic-resistant nematode strains in sheep and ibex to determine the role of transhumant sheep in contaminating alpine pastures, and whether ibex may play a role in the circulation and maintenance of anthelmintic resistant nematodes"

17. Finally, I would have found useful to add a point of discussion on the interest of exploring more widely the hosts possibly involved in the transmission and maintenance of nematodes, such as goats (see above) but also of other wild ungulates. The chamois is mentioned in line 122 but not later, although its role could also be questioned (densities sometimes high, spatio-temporal use of pastures different from that of ibex populations and certainly variable between sites).
We fully agree with the point raised by the reviewer, and previously answer to this concern. See our response to comment 12.

Recommender

I have carefully read both the manuscript and the reviewers reports and agree that this work is orginal and contributes to our understanding of parasite transmission at the domestic/wildlife interface and the circulation of drug resistance. In addition to the reviewers remarks, which I found well-justified, the authors should also mention some elements of the nematode life cycle more explicitly (for example, how long do worms live within a host?). In addition, some discussion on possible facilitation/exclusion dynamics should be considered. For example, could infection by one species of nematode in a host individual exclude infection by another? This could be particularly important to consider in terms of resistant strains - if susceptable strains outcompete resistant strains in the absence of drugs, susceptible strains could prevent infection by resistant strains post-exposure. Finally, I have made a series of detailed remarks directly on the manuscript to correct english and awkward sentences; please go over these carefully. There are also some additional content remarks which should be considered during revision.

We warmly thank the recommender Dr Karen McCoy for her positive evaluation of our work and her useful comments that helped to improve our manuscript. Our responses to comments linked to the pdf file are presented below. Minor corrections were directly modified in the manuscript and highlighted in yellow.

18. The authors should also mention some elements of the nematode life cycle more explicitly (for example, how long do worms live within a host?

We have now added more details in the introduction about the life cycle of nematodes **L73-82**: "Ungulates are usually infected by free-living larvae of gastrointestinal nematodes when they graze pasture. Infecting larvae may survive several months in the environment depending on species and climatic conditions (O'Connor et al., 2006). Following ingestion, larvae finish their development to reach the adult stage in the digestive tract. Egg-laying occurs 2─4 weeks post infection. The duration of infection by nematodes vary depending on species but last for at least 2 months from the L3 ingestion (Deplazes et al., 2016). The larvae can arrest their development in host (hypobiosis) during harsh climatic condition, delaying the egg-laying (Deplazes et al., 2016)."

Deplazes, P., Eckert, J., Mathis, A., Samson-Himmelstjerna, G. von, Zahner, H., 2016. Parasitology in veterinary medicine. Parasitology in veterinary medicine.

O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. Vet. Parasitol. 142, 1–15. https://doi.org/10.1016/j.vetpar.2006.08.035

19. It seems strange to me that the median number is identical in all samples.

In most of the samples, the number of eggs was low. Most of the time no egg was detected on the MacMaster slide, but only on the control slide and a value of 7.5 epg was attributed to the sample following Beaumelle et al., (2021). We add **L273-282**: "The number of gastro-intestinal nematodes eggs per gram of feces (epg) was counted following a modified McMaster protocol (Raynaud et al., 1970) with a solution of zinc sulphate (ZnSO4, density = 1.36, 1/15 dilution). The eggs were counted on a McMaster slide with two chambers (theoretical sensitivity of 15 eggs per gram of faeces [epg]). We also checked for the presence of low abundant parasite

propagules with a 14 mL tube filled with the remaining solution and covered with a coverslip before centrifugation (5 min at 1200 rpm) and microscopical observation ('control slide'). We attributed the value of 7.5 epg for parasites with no egg observed on the McMaster, but at least one egg observed on the control slide (for a similar procedure see Beaumelle et al, 2021)."

20. When describing the sequencing methods, it is unclear how samples were pooled. I assume by fecal sample and that a index was added somewhere to the sample. Please clarify. L259
Primer pairs are equipped with tags (short sequence labels in the 5' end of each primer). We completed the paragraph **L322-331** : « In all PCRs, we added positive PCR controls (i.e., *Haemonchus contortus* and *Teladorsagia circumcincta* DNA extracts), negative PCR controls (distilled H_2O) and negative DNA extraction controls. All samples (including controls) were tagged with unique barcode identifiers to allow pooling into a single amplicon library (Taberlet et al., 2018), and all samples were independently amplified 4 times to ensure reliability of the sequencing. Amplifications were carried out in 96-well plates, totaling 209 ibex and sheep samples, 17 PCR positive controls, 13 PCR negative controls, 7 extraction negative controls, 30 DNA extraction controls, as well as 12 empty wells in each 96-well plates to quantify tag jumping during PCR and sequencing steps (Figure S2; De Barba et al., 2014; Taberlet et al., 2018).

21. This is somewhat confusing. Early in the methods, you state that extractions were only carried out when there was more than 20 L3. Later on in the results (line 478_479) you speak again of 209 extractions. This should be clarified.
See supplementary figure S1 in appendix, the sample size is given for each stage of protocol: After the coproculture stage, fewer than 20 L3 were recovered for 48 samples out of the 257 collected samples. We did not extracted DNA of these 48 samples as the number of larvae was too low. Then, subsequent analyses were carried out on 209 bulks of larvae.». See **L436-438**: "As a result of the low level of infestation in some samples, the number of L3 hatched from eggs were not sufficient ($n < 20$) for 48 ibex or sheep samples. These samples were not used for subsequent genetic investigations."

22. I don't understand here. Would it not be more interesting to compare plains-only versus transhumant sheep? If nematodes live over a year, studying sheep over the course of the summer might not tell you much unless you study lambs.
We agree and we replace the perspective **L631-632**: "To confirm this hypothesis, the nemabiome of transhumant sheep should be compared with the nemabiome of resident sheep that stay in farms all the year around."

23. I think that you need to mention somewhere how long infections last. Less than a year?
The duration of infection by nematodes vary depending on species but last for at least 2 months from the L3 ingestion (Deplazes et al., 2016). As larvae can survive in pasture several months and can arrest their development in host (hypobiosis), hosts can be infested by gastrointestinal nematodes throughout the year (Albery et al., 2018).
In accordance with the comment #18, we added **L74-82** in the introduction information about the life cycle of gastrointestinal nematodes.

Albery, G.F., Kenyon, F., Morris, A., Morris, S., Nussey, D.H., Pemberton, J.M., 2018. Seasonality of helminth infection in wild red deer varies between individuals and between parasite taxa. Parasitology 145, 1410–1420. https://doi.org/10.1017/S0031182018000185

Deplazes, P., Eckert, J., Mathis, A., Samson-Himmelstjerna, G. von, Zahner, H., 2016. Parasitology in veterinary medicine. Parasitology in veterinary medicine.

24. So what does this mean? The resistant mutations are unlikely maintained in ibex over long periods or time?

On the contrary, we believe that resistant nematode strains can be maintained in ibex. In the revised version of our study, we have therefore clarified this point and discussed the mechanisms that could explain the maintenance of resistant nematodes in ibex populations, **L706-714**: "Whereas sheep are generally treated just before their ascent to the mountain pastures, excretion of anthelmintic via sheep feces can occur during several days after the drug administration and the molecules degradation last days, or even months (Kolar et al., 2006). In addition, sub-lethal exposition of nematode to anthelmintic residues present in the environment may select in situ for anthelmintic resistance (Dimunová et al., 2022). Unfortunately, the level of drugs in the environment, their persistence and their spread in grazed mountainous area are totally unknown. Environmental circulation of anthelmintic residues should be investigated in further studies to understand its incidence on the presence of resistant nematodes in wildlife."

See also our response to reviewer #1's comment #3.

25. But you found T. vitrinus on shared pastures, right? So it's not this.

We were surprised by this interesting, but challenging result. We agree that this strain is certainly present in patches grazed by sheep as we sampled ibex feces in area grazed by both species. But it does not seem to infect sheep. This may be due to a higher susceptibility of ibex to this strain, and/or this strain is incapable of developing in sheep. We can also assume that this strain is very sensitive to antiparasitic treatments (in agreement with the absence of resistance to benzimidazole in this parasite species) and systematically eliminated when animals are treated in sheep farm. We replace the sentence **L805-808** by: "It is possible that this strain is adapted to ibex and incapable of developing in sheep or that this strain is highly sensitive to antiparasitic treatments used in sheep farm and systematically eliminated when sheep are treated "

26. In addition, some discussion on possible facilitation/exclusion dynamics should be considered. For example, could infection by one species of nematode in a host individual exclude infection by another? This could be particularly important to consider in terms of resistant strains - if susceptable strains outcompete resistant strains in the absence of drugs, susceptible strains could prevent infection by resistant strains post-exposure.

There are certainly interspecific interactions among nematode species within the host, which can be positive or negative (Hellard et al., 2015; Lello et al., 2004). Indeed, those interactions can have implications for the management of resistance as the elimination of a nematode species/strain may favor the demography of another nematode species/strain. However, we can speculate the competition/facilitation between nematodes to be more significant if the level of infection is high, for example limited resources (e.g., free space in the gut of hosts) lead to competition or weaken host lead to facilitation. In our study, hosts are considered abundant and level of infection were generally low in comparison with others species/sheep farms (e.g., Beaumelle et al., 2021, 2022). Consequently, interspecific interaction among nematode species within the host might not be a determinant mechanism to understand and control the resistance. We added **L784-789**: "Nematodes can negatively or positively interact within the host gut, and

interactions between species or between strains may have important implication for the selection of resistance. However, the magnitude of within-host interactions between nematode strains/species and their implication in the management of resistance remains to be determined (Hellard et al., 2015; Lello et al., 2004).”

Hellard, E., Fouchet, D., Vavre, F., Pontier, D., 2015. Parasite–Parasite Interactions in the Wild: How To Detect Them? Trends Parasitol. 31, 640–652. https://doi.org/10.1016/j.pt.2015.07.005

Lello, J., Boag, B., Fenton, A., Stevenson, I.R., Hudson, P.J., 2004. Competition and mutualism among the gut helminths of a mammalian host. Nature 428, 840–844. https://doi.org/10.1038/nature02490