Research Article

Factors influencing gastrointestinal parasites in a colony of Alpine ibex (*Capra ibex*) interacting with domestic ruminants

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Abstract

Parasitic infections in populations of wild herbivores can affect the individual fitness and population dynamics of their hosts. In this study, the ecology of gastrointestinal (GI) parasites was investigated in an Alpine ibex colony of the Marmolada massif, eastern Alps. Both individual and environmental factors were studied to reveal their relationship with parasite prevalence and burden. In a four-year period, 414 individual faecal samples of ibex were collected on a monthly basis during summer and autumn and were subjected to quali-quantitative examinations for the identification and quantification of oocysts and eggs. Domestic ungulates grazing in the same area were also sampled to investigate the risk of GI parasite transmission. Negative binomial regression models were developed to study the influence of physiological and environmental factors on parasite burdens. The results of the qualitative examination were in agreement with the few data already present in the literature for Alpine ibex, showing high prevalence values for Coccidia and GI strongyles, low values for Cestoda and sporadic presence of whipworms. Higher burdens in kids were found for Coccidia and Cestoda. Analysis of the GI endoparasite community of cattle and sheep suggested a negligible risk of parasite transmission to the Alpine ibex population. The sanitary risk represented by Coccidia and Cestoda in ibex kids and some peculiarities of the endoparasite distribution patterns in the Marmolada ibex population call for future in-depth ecological studies to investigate their influence on the limited growth rate shown by the ibex colony during the last decade.

Introduction

Gastrointestinal (GI) parasites have a major role in the life history and population dynamics of domestic and wild herbivores (Stien et al., 2002; Holmes, 1982). Based on the level of parasitic burden, parasites can influence body growth, reproductive performance, and the survival of both offspring and adults (Aleuy et al., 2018; Irvine et al., 2006; Gunn and Irvine, 2003; Albon et al., 2002; Roberts et al., 2002). Different factors can determine parasite distribution in the host population and consequently the parasitic burden of individual hosts. Individual characteristics such as host age, sex and behaviour have a major impact (Wilson et al., 2002). When parasites are acquired from the environment over time, an increase in parasite load with age is expected (Wilson et al., 2002). However, for some parasites (e.g., Coccidia), acquired immunity plays an important role, causing a higher resistance to infection in adult animals. Consequently, young animals usually have higher burdens of these parasites (Kołodziej-Sobocińska et al., 2019). Males seem to be more susceptible to parasitic infections than females, which is probably due to their higher concentration of testosterone, which predisposes them to greater immunosuppression (Ferrari et al., 2010). Furthermore, vertebrates have developed many behavioural strategies to reduce the contact rate with the infective stages of parasites or their vectors, such as the spatial segregation of feeding and defecating areas (Brambilla et al., 2013). If individuals differ in their behaviour, this can generate heterogeneities in parasitism rates (Wilson et al., 2002).

On the other hand, the survival of free-living stages and the abundance of invertebrate intermediate hosts are influenced mostly by climatic and environmental characteristics, such as temperature, humidity and rainfall (Leathwick and Sauermann, 2018). Medium-high temperatures are usually a positive factor for the sporulation of coccidia and for the hatching of eggs and the subsequent development and survival of infective GI strongyl larvae (Rose et al., 2015; Ruiz et al., 2006), although they are dependent on the specific environmental context. Another important factor is UV exposure. Direct exposure to sunlight damages the DNA of coccidia oocysts (Lassen et al., 2013) and reduces the survival of nematode larval stages (Knapp-Lawitzke et al., 2016; Rose et al., 2015). Many authors took into consideration the seasonal patterns of parasite infection, considering at the same time different environmental factors (e.g., temperature, humidity and precipitation). In the alpine environment, the summer-autumn period is associated with an increase in the prevalence of both Coccidia and Nematoda, which conversely decrease in winter (Walker et al., 2017).

The interaction with other host species (e.g., sharing of pastures) can also influence the dynamics of wild ruminant parasites. Parasites can be classified as generalists or specialists. Specialist parasites generally infest a single host species, whereas generalist parasites have evolved to survive and reproduce in more host species (Agosta et al., 2010). The available literature about parasite transmission between wildlife and livestock is limited, and the impact of interactions with livestock on wildlife health status can vary according to the geographical area and the rangeland management system (Walker and Morgan, 2014; Zaffaroni et al., 2000). Obviously, generalist parasites seem to be the ones with the greatest health significance for wildlife (Zaffaroni et al., 2000). Among these, *Haemonchus contortus* and *Trichostrongylus axei* have been detected in several host ruminant species grazing in the same area (Citterio et al., 2006; Soulsby, 1982).

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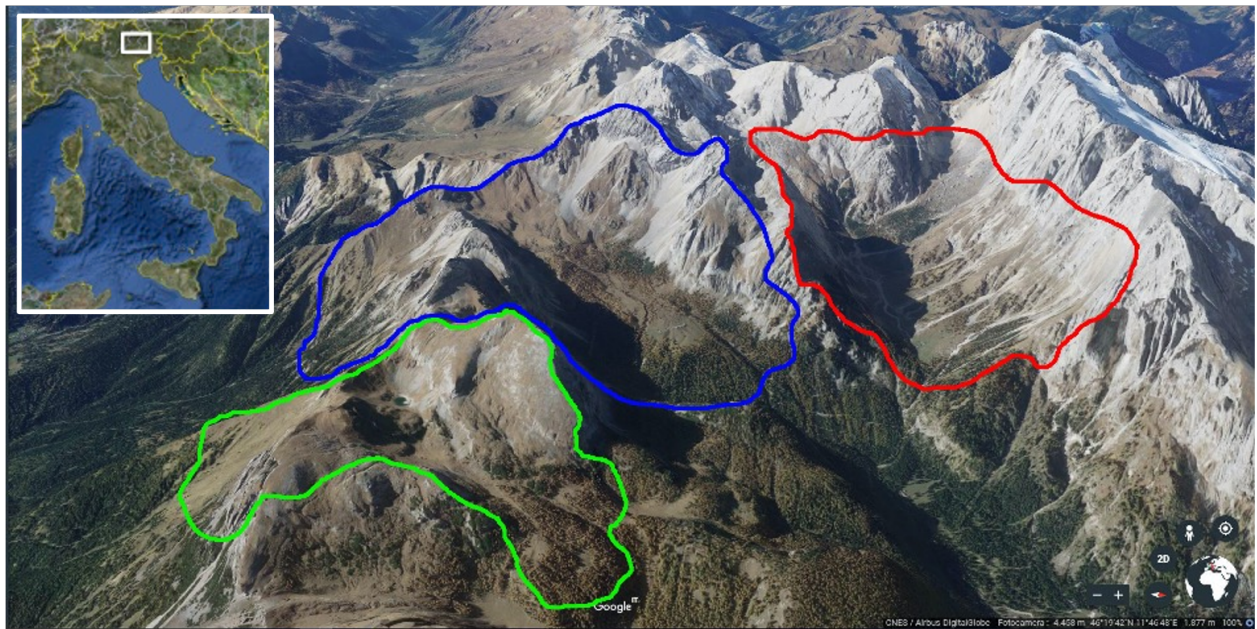
Figure 1 – Study area and subunits. Green outline: Lago di Negher (LA); blue outline: Franzedas/Cime d'Auta (FR/CI); red outline: Ombretta/Ombrettola (OM).

During the second half of the last century, the abandonment of agriculture in the Italian Alps, as well as in other marginal areas, has allowed a massive natural reforestation of meadows and pastures (Cocca et al., 2012; Falcucci et al., 2007). This re-wilding has favoured range expansion and an increase in the population size and density of wild ungulates (Carnevali et al., 2009), which increase the possibility of contact between domestic and wild ungulates. In addition, thanks to reintroduction projects, the richness of wild ungulate species has also been locally increased. This is especially the case for the Alpine ibex, which has been successfully reintroduced in different areas of the Eastern Alps. It is worth noting that Alpine ibex in Italy benefited from the status of being considered a specially protected species and therefore were never subjected to hunting activity, apart from the territory of the autonomous province of Bolzano (outside the study area), where local administration authorized minor harvests of the species as control interventions (Carnevali et al., 2009).

The aim of this research was to investigate the influence of different factors on the epidemiology of GI parasite infection in the colony of the Alpine ibex of the Marmolada massif, eastern Alps. The influence of the interaction with domestic ungulates (cattle and sheep) grazing in the same area was also assessed.

Materials and methods

Study area and animals

The study area is situated in the Marmolada massif group in the Eastern Italian Alps (Dolomites, 46°26′13″ N, 11°51′54″ E), which is characterized by high elevation (1400 m–3343 m a.s.l.) and narrow valleys with steep slopes, delimited by rocky ridges and mountain peaks that can rise above 3000 m a.s.l. The climate is typically alpine, with a mean annual temperature of 1.7 °C and average monthly temperatures varying from approximately −5 °C in December-January to ap-

proximately 10 °C in July-August. The total yearly precipitation is approximately 1400 mm, and snow cover in the area used by the Alpine ibex (see below) may last from late October to early June. The average temperature and precipitation were different in the four years of the study (Tab. 1), with the highest average temperature and the lowest overall precipitation values in 2015. These parameters were recorded by two different weather stations of the Regional Environmental Agency (ARPAV: https://www.arpa.veneto.it/bollettini/meteo60gg/Mappa_TEMP.htm?t=RG). Daily precipitation (mm) data were recorded by the "Malga Ciapela" meteorological station (station n. 11), the nearest to the study area, although at a lower altitude (1474 m a.s.l.). Average daily temperatures (T°) in °C were recorded by the "Monti Alti di Ornella" (station n. 382), located at 2227 m a.s.l., in the middle of the altitudinal range of the study area, and 6 km from it.

The local ibex colony (Scillitani et al., 2012) currently ranges over approximately 65 km² across an altitudinal range from 1700 m to 2900 m a.s.l. The colony was founded in 1978–1979, with the reintroduction of 10 individuals from Gran Paradiso National Park (western Italian Alps). It reached its maximum range and size in 2002, with more than 450 individuals. Later, the colony was decimated by an outbreak of sarcoptic mange that occurred in 2003 and 2004. Between 2004 and 2005, adult ibexes (n=54) were captured and treated with ivermectin-based drugs to help address the mange outbreak. In addition, between 2006 and 2007, 14 adult males from the colony of Jof-Fuart Montasio (Tarvisio) were translocated thanks to a restocking programme (Scillitani et al., 2013). After a minimum estimated size of approximately 100 individuals in 2006, the population subsequently increased to an estimated size of almost 300 in 2019 (Stefano Vendrami, *pers. comm.*). Although before the mange outbreak, the population had been increasing at a yearly rate of approximately 21%, it recovered afterwards at a much slower rate of approximately 6–8%, and observations of marked females indicate a kid/female ratio of 0.46 (Maurizio Ramanzin, *pers. comm.*).

Alpine ibexes are characterized by sexual and social segregation between the two sexes, and outside the reproductive period, mixed groups are rare (Grignolio et al., 2007). This division was also confirmed in the Marmolada massif by the analysis of the VHF and GPS data and by the observation of the ear tags of some animals that have been radio-collared and marked (Scillitani et al., 2013). Three population subunits were identified for the study (Fig. 1) based on topographic features and sexual segregation that reduce the exchange of individuals (Scillitani et al., 2012). Two subunits are used in spring–summer mostly by females and seldom by males (Ombretta and Ombrettola valleys,

Table 1 – Climatic data for the four study years (SD=Standard Deviation).

Year	Temperature	Precipitation
	Yearly average(°C)(±SD)	Overall(mm)
2013	1.58 (-5.42–8.58)	1453.6
2014	2.45 (-3.18–8.08)	1801.6
2015	3.14 (-3.65–9.94)	941.0
2016	2.20 (-4.23–8.64)	1344.2

Table 2 – Number of faecal specimens collected from Alpine ibex for each subunit (FR-CI=Franzedas valley and Cime d'Auta; LA=Lago di Negher; OM=Ombretta and Ombrettola valleys).

Year	Population counts (N)[1]	FR-CI				LA				OM				Total by year
		K	F	M	nd[2]	K	F	M	nd[2]	K	F	M	nd[2]	
2013	224	8	63	0	16	0	0	42	0	9	33	0	8	**179**
2014	178	8	17	1	5	0	0	24	0	3	14	3	14	**89**
2015	201	10	23	3	1	0	0	26	0	2	7	7	8	**87**
2016	233	2	12	2	7	0	0	18	0	0	10	4	4	**59**
Total by sex and age-class		**28**	**115**	**6**	**29**	**0**	**0**	**110**	**0**	**14**	**64**	**14**	**34**	
Total for sub-units			**178**				**110**				**126**			**414**

[1] These numbers refer to the overall number of individuals in the whole Marmolada area from yearly block counts.

[2] nd=not determined (samples belonging to yearlings, sub-adults or adult females for which it was not possible to determine the specific individual from which the sample originated).

hereafter named OM, and Franzedas valley and Cime d'Auta, hereafter named FR-CI), and one subunit is used intensively by males and seldom by females (Lago di Negher, hereafter named LA). This subdivision is simplified with respect to that of a previous study (Cassini et al., 2015) because we merged Franzedas and Cime d'Auta since recent GPS telemetry data indicate that ibex females actually move between these areas during summer (Semenzato et al., 2021).

Other wild ungulates present in the area are Alpine chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and mouflon (*Ovis aries*). However, only Alpine chamois can substantially overlap their range with that used by ibex, while the other species remain mostly at lower elevations, and their spatial overlap with ibex is negligible. Part of the high elevation pastures in the study area are grazed by domestic cattle and sheep during summer and early autumn (June-September), which may cause a spatial overlap with the lower portion of the range used by ibex, especially for sheep. Two dairy cattle herds graze the Alpine pastures of the Ombretta (OM area) and Franzedas valleys (FR-CI) during late June-mid September, while a transhumant sheep flock (approximately 1000 heads) grazes in the area for limited periods (one-two weeks) during summer, moving each year through LA and FR-CI.

Sampling methods

In total, 414 ibex faecal specimens were collected on a monthly basis from June to November (the period of accessibility of the study area) from 2013 to 2016 (Tab. 2). During the sampling period, ibex typically maintained a sexual segregation pattern, with adult males clustering in a single subunit (LA) and many separated groups of females with yearlings and kids (rarely also young males up to 3 years) occurring in the other two subunits. Smaller groups of adult males were rarely encountered and therefore sampled in the FR-CI and OM areas.

The areas of the three subunits were periodically walked during the June-November periods of 2013, 2014, 2105 and 2016, tentatively on a monthly basis. Each "female" group (including kids, yearlings and occasionally young males less than three years old) and "male" group (including only males of variable age) were examined with a $60\times$ magnifier telescope (CT85, Swarovski, Absam, Austria) to count the number of individuals and identify their sex (male or female) and age. After seeing one or a few individuals defecating, the faeces were collected immediately after the group had moved away. It was not possible to assign each faecal sample to a specific individual among the animals previously observed. Therefore, we used only two age classes (kid: <1 year old, "adult": $\geqslant$1 year old), according to the size of faecal pellets. Sex was not determined when yearlings (1–2 years) were present in mixed groups with females. We collected few samples from each group, and we are confident that the samples did not originate from the same animal.

Domestic ungulates were also sampled between 2015 and 2018. Of these, 53 specimens were retrieved from cattle and 48 from sheep. Faecal specimens were collected through rectal inspection from 8 in-

dividuals in each of the two herds of cattle grazing in the study area (approximately 40 adult females in FR-CI and approximately 25 adult animals in OM) at the beginning (late June) and at the end (mid-September) of the grazing period in 2015 and again from 10 and 11 animals in the OM group, in early July and mid-September of 2017 respectively. Ovine faecal specimens were obtained from one transhumant flock moving through FR-CI and LA subunits for approximately two weeks every year in the first half of July. In the study area, transhumant flocks live externally on pasture year round, moving permanently through the whole territory of the northeastern Italian regions, passing cold periods (October-March) in the lowlands, and warmer periods (April-September) in the highlands. The flock was first sampled in June 2016 (n=12) through collection of faecal specimens from soil, immediately after defecation, and subsequently monitored with three more samplings at different times of the year, in September 2017 (n=12), October 2017 (n=13) and in January 2018 (n=11). Data and information on general management and antiparasitic treatments were collected during the visits.

All specimens were stored at a refrigerated temperature until the time of analysis (maximum 5 days after collection).

Laboratory analysis

A standardized quantity (5 g for cattle, 2 g for other species) of each sample underwent a qualitative test by flotation in a solution of $NaNO_3$ and sucrose with a specific density of 1300 to search for the following parasites: Coccidia, Gastro-Intestinal Strongyles (GIS), *Nematodirus/Marshallagia*, *Trichuris* spp., *Capillaria* spp. and Cestoda. The number of eggs per gram (EPG) and oocysts per gram (OPG) were determined by the McMaster technique using the same solution and a quantity of faeces equal to the previous test. The specimens that tested positive for qualitative analysis but negative for the McMaster technique were classified as subthresholds and were arbitrarily assigned a value corresponding to the mean between 0 and the threshold value. We decided to use these quantitative output values (expressed in OPG/EPG) as a proxy of parasite abundance, since this is the only quantitative data retrievable in studies based on coprology.

In addition, specimens collected from ibex during different months in 2013 and coming from different subunits were pooled together and analysed by means of coproculture with the aim of identifying the genus of L3 larvae of the GI strongyles. This technique consisted of diluting the faeces in mineral water and vermiculite. Subsequently, the mixture was kept for one week in a thermostat at 27 °C. The faeces were placed in a Baermann apparatus to allow the migration of the L3 larvae that developed during the incubation. Vital larvae were placed on a slide and analysed under a microscope at 400×. A total of 412 larvae were identified according to morphological keys (Van Wyk and Mayhew, 2013; MAFF, 1986). As per the diagnostic identification procedure at the Laboratory of Parasitology of the University of Padova and due to the difficulties in confident differentiation of some genera, third-stage larvae were clas-

Table 3 – Prevalence and abundance values of the different parasites of Alpine ibex, cattle and sheep, considering all collected samples (SE=Standard Error).

Parasite	Alpine ibex (n=414)		Cattle (n=53)		Sheep (n=48)	
	Prevalence N pos(%)	Abundance Mean OPG/EPG (SE)	Prevalence N pos(%)	Abundance Mean OPG/EPG (SE)	Prevalence N pos(%)	Abundance Mean OPG/EPG (SE)
Coccidia	414 (100%)	3169.7 (773.8)	11 (20%)	8.3 (2.8)	46 (96%)	1389.1 (302.2)
GI Strongyles	408 (98.6%)	261.5 (15.1)	48 (90%)	20.8 (3.3)	44 (92%)	277.1 (85.5)
Nematod./Marsh.	298 (72.0%)	19.7 (0.7)	3 (6%)	0.9 (0.6)	23 (23%)	14.6 (2.8)
Cestoda	78 (18.8%)	37.3 (16.4)	6 (11%)	1.3 (0.5)	9 (19%)	121.9 (89.6)
Trichuris spp.	11 (2.7%)	1.4 (0.5)	0	0	4 (8%)	2.6 (1.3)
Capillaria spp.	2 (0.5%)	0.1 (0.1)	0	0	1 (2%)	0.5 (0.5)

sified as belonging to 4 main morphotypes attributable to the following genera/groups of genera: *Haemonchus* (16 intestinal cells, sheath tail length about 75 μm with short filament); *Trichostrongylus/Teladorsagia/Ostertagia/Marshallagia* (16 cells, sheath tail length 30 μm–50 μm without filament); *Oesophagostomum/Chabertia* (18–32 cells, sheath tail length 120 μm–150 μm with long filament); *Bunostomum* (small larva, oesophagus with a prominent single bulb caudally, sheath tail length about 120 μm with medium-long filament).

Data analysis

Prevalence and abundance values were displayed using simple descriptive statistics for each parasitic group of all investigated hosts. A multivariate generalized linear model (specifically a negative binomial regression) was used to analyse the quantitative results of Alpine ibex copromicroscopy for each parasitic group. For this model, group, year and month were used as independent factor variables together with a new variable, named physiological class, joining sex and age with three possible values (kid, female older than 1 year, male older than 1 year), to avoid excluding all kids from the analysis due to their unidentified sex. Because the purpose of the model was to evaluate the epidemiological influence of the independent variables on parasites, all the variables were maintained in the final model; this approach makes it possible to control for possible confounding effects linked with the observational study design.

Differences among samplings were also evaluated for cattle and sheep using a univariate negative binomial regression model: differences between all animals sampled at the beginning (June-July) and at the end (September) of the alpine pasture season were considered for cattle, whereas differences among each sampling along the whole monitoring period were analysed for sheep. Statistical analyses were conducted in STATA® 12.1 (StataCorp LLC, College Station, TX, USA).

Results

### Parasitological results in Alpine ibex and domestic ruminants

The prevalence and abundance of different parasites in the Alpine ibex, cattle and sheep are reported in Tab. 3. Coccidia and GI strongyles were prevalent and abundant in both ibex and sheep, whereas cattle showed negligible burdens for these parasites. Cestoda were also found in all species; instead, *Trichuris* and *Capillaria* were sporadically encountered only in ibex and sheep.

As per the identification of strongylid L3 in the ibex population, out of 412 identified larvae, 234 (56.8%) were attributable to the genera *Trichostrongylus/Teladorsagia/Ostertagia/Marshallagia* and 178 (43.2%) to the genera *Oesophagostomum/Chabertia*. None of the analysed specimens were ascribable to *Haemonchus* or to *Bunostomum*.

Cattle herders reported that their animals were kept as a unique herd during summer grazing but gathered from different farms. The reported anti-parasitic protocols ranged from absence of any pharmacological intervention, treatment of clinical cases only or anthelmintic treatment of the whole herd in autumn, after pasture period. However, parasitic burden was similarly low both at the beginning and at the end of the

grazing season, with only a significant decline (*p*<0.01) in strongyles abundance (Fig. 2).

The sheepherder referred to treating the whole flock twice a year, in early spring (March-April) and in autumn (September-October) with macrocyclic lactone or benzimidazolic drugs. Consequently, strongyles showed a sharp decline after the treatment in late September and a higher value in the following January (Fig. 3). No treatment was mentioned for Coccidia, which showed a fluctuating trend with significant differences among samplings (Fig. 3).
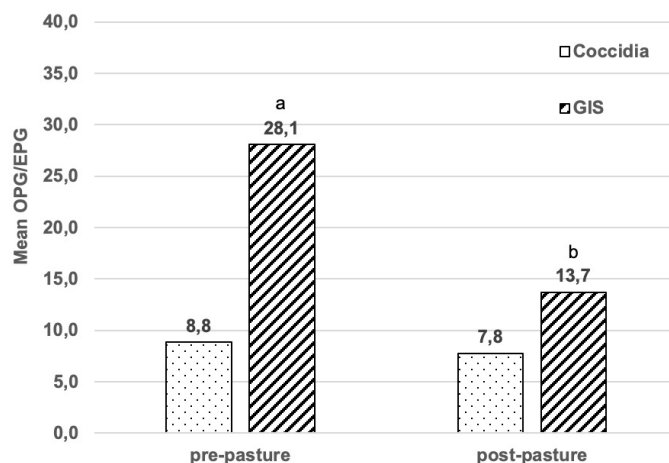
Figure 2 – Coccidia and gastrointestinal strongyle (GIS) abundance values (mean OPG/EPG) at the beginning (pre-pasture, n=26) and end (post-pasture, n=27) of the summer pasture period in cattle. Different letters at the top of the bar show significant differences between pre- and post-pasture for each parasitic group.
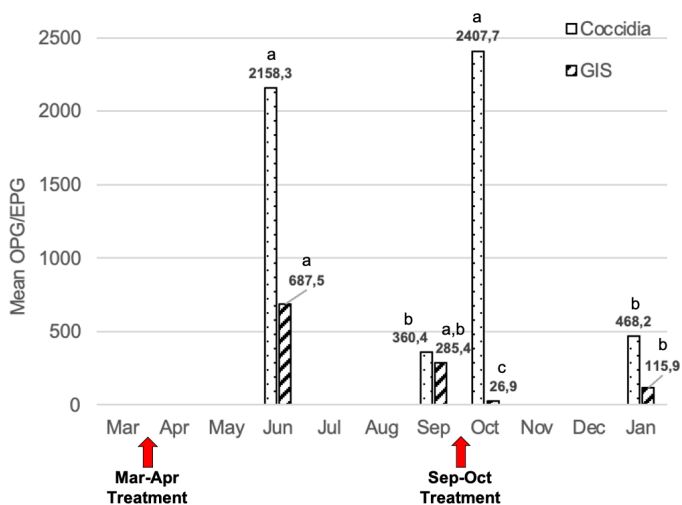
Figure 3 – Coccidia and gastrointestinal strongyle (GIS) abundance values (mean OPG/EPG) at different samplings (June 2016, n=12; September 2017, n=12; October 2017, n=13; January 2018, n=11) in the sheep flock. Different letters at the top of the bar show significant differences among samplings for each parasitic group. The treatments are highlighted by a red arrow.

Table 4 – Abundance and prevalence of Coccidia, GI strongyles, Cestoda and *Nematodirus/Marshallagia* in the Alpine ibex population (n=414) (SE=Standard Error; N/D=not determined).

Factors	Levels	N	Coccidia Abundance Mean OPG (SE)	GIS Abundance Mean EPG (SE)	Cestoda Abundance Mean EPG (SE)	Prevalence	*Nematodirus/Marshallagia* Abundance Mean EPG (SE)	Prevalence
Age/Sex	Kid	42	12373.6 (7426.0)	168.8 (24.8)	189.3 (156.9)	28.6%	22.6 (2.7)	78.6%
	F	179	2144.8 (173.6)	297.2 (27.6)	19.0 (4.5)	17.9%	20.0 (1.0)	73.7%
	M	130	1536.7 (143.5)	207.1 (16.6)	11.9 (4.8)	13.1%	19.6 (1.3)	73.1%
	N/D	63	3315.1 (676.0)	333.7 (43.9)	40.5 (18.0)	27.0%	17.1 (1.9)	60.3%
Group	FR-CI	178	4430.9 (1759.3)	270.4 (28.1)	32.0 (7.9)	24.7%	18.8 (1.1)	69.1%
	LA	110	1621.8 (165.7)	191.6 (15.9)	13.6 (5.6)	13.6%	20.0 (1.4)	73.6%
	OM	126	2739.2 (486.7)	309.9 (25.4)	65.5 (52.4)	15.1%	20.6 (1.2)	74.6%
Year	2013	179	4301.4 (,753.5)	292.8 (27.5)	26.5 (5.1)	25.7%	21.9 (0.9)	81.0%
	2014	89	1807.3 (153.4)	238.8 (26.9)	24.4 (12.5)	19.1%	18.8 (2.0)	62.9%
	2015	87	2192.0 (493.9)	210.3 (24.9)	87.9 (75.9)	12.6%	19.8 (1.4)	73.6%
	2016	59	3233.1 (702.2)	275.8 (34.2)	14.8 (10.0)	6.8%	14.0 (1.6)	55.9%
Month	June	34	1945.6 (227.8)	265.4 (54.0)	5.9 (5.9)	2.9%	17.6 (2.2)	67.6%
	July	73	3409.2 (622.1)	319.5 (37.2)	45.5 (15.8)	38.4%	16.4 (1.8)	57.5%
	August	84	5344.6 (3697.9)	255.7 (33.0)	21.4 (7.1)	20.2%	19.9 (1.8)	70.2%
	September	104	2494.5 (447.8)	319.0 (38.9)	86.1 (63.7)	17.3%	19.5 (1.2)	75.0%
	October	70	2125.6 (223.9)	218.5 (22.1)	2.5 (1.5)	5.7%	23.2 (1.8)	82.9%
	November	49	2858.2 (861.0)	121.4 (14.0)	20.4 (7.8)	20.4%	20.9 (1.7)	77.6%

Factors influencing parasite burden in ibex

Abundance and prevalence values for the different parasitic groups in relation to the considered grouping variables are shown in Tab. 4. Prevalence is not displayed for Coccidia and GI strongyles, in consideration of their uniformly very high values (Tab. 3).

The results of multivariable analyses are reported in Tab. 5 for coccidia, GI strongyles and Cestoda. Major significant results were as follows: kids were more heavily infected with Cestoda and Coccidia, while adult females were more heavily infected by GI strongyles. Ibexes from OM were less infected by Coccidia and more infected by GI strongyles than those from FR/CI. Regarding the yearly comparison, 2014 and 2015 showed significantly lower GI strongyle and coccidia burdens than 2013. No significant difference emerged for *Nematodirus/Marshallagia*.

Discussion

The aim of this study was to study the epidemiology of GI endoparasites in the Alpine ibex colony living in the Marmolada massif ecosystem exposed to interactions with domestic ruminant populations (sheep and cattle). The Alpine ibex population was sampled throughout four subsequent years, investigating the influence of individual factors through the comparison of three physiological classes based on sex and age (kid, female older than 1 year, male older than 1 year). Differences among subunits were associated with the presence of different species of domestic ruminants, whereas differences among years were interpreted on the basis of two environmental variables (temperature and precipitation), which are usually considered highly relevant for the external phase of endoparasites (Leathwick and Sauermann, 2018).

The GI endoparasite community of the Alpine ibex colony of the Marmolada massif was similar to that already reported in the western Alps (Marreros et al., 2012), with very high prevalence values for Coccidia (100%) and GI strongyles other than *Nematodirus/Marshallagia* (98.6%), high prevalence for *Nematodirus/Marshallagia* (72.0%), low values for Cestoda (18.8%) and sporadic presence of whipworms (*Trichuris* and *Capillaria* genera), although the methodology used may lack in sensitivity for Cestoda. The prevalence values recorded in the present study are very similar to those in the study of Marreros et al. (2012), which is, to our knowledge, the only published study on Alpine ibex GI parasites conducted through coprological analyses. It is not possible to compare our data on parasite abundance, since in the cited study, only semiquantitative results were displayed; therefore, our research is the first to provide abundances in terms of mean oocyst/egg

output for different parasite groups in Alpine ibex. In addition, we tried to provide the relative percentage of strongylid genera through identification of L3 at the genus level after coproculture. The results of this analysis indicate that the *Haemonchus* and *Bunostomum* genera did not circulate in the Marmolada ibex population, which is in agreement with data provided by studies conducted through adult parasite retrieval and identification (Zaffaroni et al., 2000; Balbo et al., 1978). Instead, the genera associated with the two larval morphotypes identified in our samples contain the species previously identified as frequent (Balbo et al., 1978) and/or dominant (Zaffaroni et al., 2000) in *C. ibex* gastrointestinal helminthic fauna (i.e., *Marshallagia marshalli*, *Teladorsagia circumcincta*, *Trichostrongylus axei*, *Chabertia ovina*).

Regarding individual factors, significant differences were found mostly between kids and adults. It is worth noting that in our study, we were able to include a relevant number of faecal specimens belonging to the kid category, which is not easily achieved in this kind of study (e.g., only 2 samples were collected by Marreros et al., 2012). The mean outputs of Coccidia oocysts and Cestoda eggs were both significantly higher in kids than in adults. This is probably because these two groups of parasites induce important host immune responses, causing an early peak in the age-intensity curve (Wilson et al., 2002). This means that very young individuals present a higher parasite burden due to their immunological response still being under development. Although many other biotic and abiotic factors could also be involved, the importance of coccidia and Cestoda in worsening the health status of kids should be considered when analysing the demographic parameters of the whole population. In contrast, adult females were significantly more infested with GI strongyles than kids, which is in line with age-intensity curves observed when immunity plays a minor role and parasites are acquired from the environment over time, with increased abundance with host age (Wilson et al., 2002). This kind of age-intensity pattern is usually not found for strongyles in wild bovids (Zaffaroni et al., 1997; Citterio et al., 2006; Marreros et al., 2012), while it is more frequent in wild cervids (Zaffaroni et al., 1997; Milner et al., 2013). Among adults, males seem to have a lower parasitic burden than females, which is different from the literature data. Previous studies on both Alpine ibex and chamois (Decristophoris et al., 2007; Hoby et al., 2006) reported that male subjects have a higher parasitic load, probably due to the effect of testosterone. The differences with our study could be related both to the use of coprological analysis instead of parasite isolation or to ecological and biological peculiarities of the reintroduced Marmolada ibex population. The use of a multivariable model allowed us to control the effect of area, year and month; therefore, the results related

Table 5 – Results of multiple analyses (negative binomial regression) for Coccidia, GI strongyles and Cestoda in Alpine ibex (n=351). The *p*-value of significant differences is in bold; *"ref"* indicates the reference category.

Factors	Levels	N	Coccidia Coef. (95% C.I.)	*p*-value	GI strongyles Coef. (95% C.I.)	*p*-value	Cestoda Coef. (95% C.I.)	*p*-value
Age/Sex	Kid	42	*ref*		*ref*		*ref*	
	F	179	-1.601 (-1.931 – -1.269)	**<0.001**	0.466 (0.133 – 0.798)	**0.006**	-3.085 (-5.430 – -0.740)	**0.010**
	M	130	-2.105 (-2.634 – -1.575)	**<0.001**	0.395 (-0.159 – 0.948)	0.162	-4.039 (-8.017 – -0.062)	**0.047**
Group	FR-CI	149	*ref*		*ref*		*ref*	
	LA	110	0.178 (-0.318 – 0.675)	0.482	-0.040 (-0.577 – 0.496)	0.883		*p*>0.05[1]
	OM	92	-0.300 (-0.558 – -0.041)	**0.023**	0.445 (0.179 – 0.711)	**0.001**		
Year	2013	155	*ref*		*ref*		*ref*	
	2014	70	-0.354 (-0.642 – -0.065)	**0.016**	-0.533 (-0.837 – -0.229)	**0.001**		*p*>0.05[1]
	2015	78	-0.556 (-0.837 – -0.274)	**<0.001**	-0.295 (-0.586 – -0.003)	**0.047**		
	2016	48	0.036 (-0.305 – 0.377)	0.834	-0.221 (-0.593 – 0.151)	0.244		
Month	June	28	*ref*		*ref*		*ref*	
	July	37			0.462 (-0.053 – 0.977)	0.079		
	August	76		*p*>0.05[1]	0.306 (-0.184 – 0.797)	0.221		*p*>0.05[1]
	September	103			0.534 (0.083 – 0.984)	**0.020**		
	October	66			0.359 (-0.142 – 0.861)	0.160		
	November	41			-0.340 (-0.865 – 0.185)	0.204		

[1] Differences among all variables were not significant

to the physiological class cannot be attributed to these variables or to the unbalanced sampling in the different areas.

The influence of domestic ungulates (sheep and cattle) on ibex endoparasite dynamics was indirectly investigated, assessing potential differences between subunits characterized by the presence of two domestic species with different patterns: only sheep in LA, sheep and cattle in FR-CI, and only cattle in OM. In addition, the parasitic status of the sheep and cattle was evaluated through coprological analyses, sampling the animals at different times throughout the year.

The results of these analyses showed that cattle herds had low burdens for all considered parasitic groups. More interestingly, parasitic load was not increased at the end of the alpine pasture period but was similar to, or even lower in the case of GI strongyles, that at the beginning. This finding is probably due to an optimal use of pasture, as already suggested (Martini et al., 2009). Moreover, in agreement with the literature, no coccidian species and very few nematodes were reported to be in common between cattle and Alpine ibex (Winter et al., 2018; Taylor et al., 2015; Zaffaroni et al., 2000). In consideration of what was reported above, we can state that the probability of endoparasite transmission between cattle and ibex is very remote in the Marmolada area; therefore, cattle do not represent a sanitary risk.

In contrast to cattle, the abundance and prevalence values of most parasites in sheep were high and similar to those found in the ibex population. The burden of the two most important parasitic groups (Coccidia and GI strongyles) in sheep showed a fluctuating trend, which may be partly due to management protocols (e.g., GI strongyles were strongly reduced by anthelmintic treatment) and partly to environmental drivers (e.g., differences in soil and climatic characteristics of high- and low-land areas). Nevertheless, considering the practice of treating sheep flocks twice a year, i.e., in March-April and in September-October, and based on the results of the first sampling conducted in June 2016, we can assume that sheep usually presented a high strongyle burden during their passage through the study area (end of June-early July). In addition, different helminth species are common between sheep and Alpine ibex (Zaffaroni et al., 2000), which implies that ovines can represent a potential risk for endoparasite transmission. Notwithstanding this potential risk, a higher GI strongyle burden was found in the OM subunit, which was the only group not sharing pasture with sheep. In addition, one of the more threatening generalist species (*H. contortus*), which is typically found in sheep, was absent in the whole ibex population, suggesting a very limited probability of nematode cross-transmission through pasture sharing in the Marmolada area. No sig-

nificant differences among subunits were found for Cestoda, whereas the FR/CI group reported the highest parasitic burden for Coccidia. Notwithstanding the higher infestation in this group (that is sharing pasture with sheep), it should be noted that there is only one species of *Eimeria* (*E. ovis*, also named *E. bakuensis*) in common between sheep and ibex (Khodakaram-Tafti and Hashemnia, 2017; Perez et al., 2006), and it is rarely encountered in Europe. Consequently, the risk for coccidian transmission from sheep to ibex is also limited.

The influence of environmental variables on parasite dynamics was difficult to investigate due to their complex interactions. In our study, we monitored the temporal trends of parasitic outputs to assess whether evident changes in abundance of each parasitic group were present. Unfortunately, due to adverse winter weather conditions, it was not possible to monitor the parasitic status of the animals throughout the year but only in the warmer months (June-November); therefore, we could not appropriately investigate seasonal trends. However, small differences among monitored months were found only for GI strongyles.

As per differences among years, the coccidia and GI strongyle emissions in 2014 and in 2015 were significantly lower than those in 2013. It is worth noting that 2015 showed the highest temperature and the lowest precipitation values (Tab. 1), suggesting that these parasites may be sensitive to dry and hot conditions in an alpine environment, as already proposed (Walker et al., 2017; Knapp-Lawitzke et al., 2016; Khadijah et al., 2013; O'Connor et al., 2006; Rose, 1963). However, 2014 showed similar environmental conditions to 2013 and lower outputs of Coccidia and GI strongyles, confirming that the influence of temperature and precipitation on parasite dynamics is difficult to assess in a complex multifactorial natural ecosystem.

Conclusions

We attempted to analyse the influence of different factors on GI endoparasite dynamics in a specific ecosystem where wild and domestic animals share the same pasture. Our study was limited by the impossibility of sampling ibex during cold months due to the inaccessibility of the area, and consequently, the obtained picture is to some extent only partial.

The ibex population in the Marmolada massif ecosystem presented a sufficiently rich endoparasite community, with evident differences in abundances between adults and kids, with a large number of the latter category being investigated for the first time. The presence of sheep and cattle does not seem to influence the ibex endoparasite community,

suggesting that a limited use of mountain pasture by domestic animals may not represent a parasitological threat to wild ruminants. Temporal trends were difficult to analyse because of the abovementioned sampling limitation.

Coccidia and cestodes were more abundant in kids, and this might represent a potential threat to their health status and survival. Therefore, these parasites might also play a role in the slow growth rate of the Marmolada alpine ibex colony during the last decade. However, the lower abundance of this parasite in adults suggests a good immunological response at the population level. The results regarding gastrointestinal strongyle abundance in age and sex classes suggest that the Marmolada population could be unique and worth future in-depth ecological studies. ✍

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